HLA Barrier In Renal Transplantation : Pre& Post

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Introduction

The presence of human-leukocyte antigen (HLA)- antibodies remains a large barrier to kidney transplantation leading to increased morbidity and mortality on the transplant waiting list.

Over the last decade a number of new approaches were developed to overcome these barriers.

De novo DSA risk reduction is essential to prevent chronic AMR and improves long-term graft survival in all solid organ transplant patients.

Utilization of solid-phase single-antigen assays as part of risk-based monitoring for circulating DSA has increased.

Questions To Answer

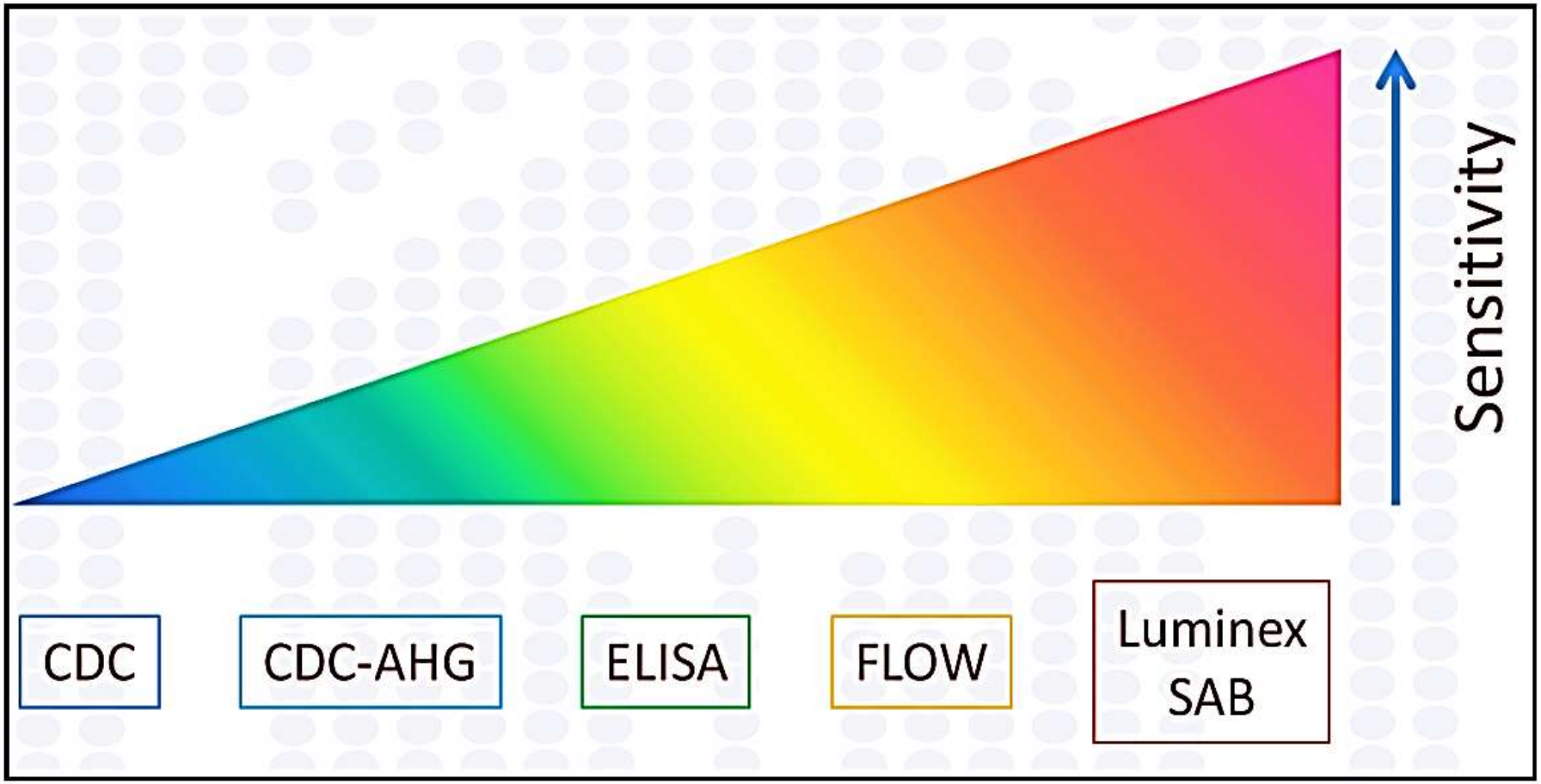
- How to avoid ?
- What is its clinical relevance ?
- How to monitor ?
- How to manage ?
- And more …

The Highly Sensitized Patient?

"Potential recipient of a graft with an amount of circulating anti-HLA antibodies which limits "notably" his/her access to transplantation".

Spain	**PRA CDC >90% (only1 of 10 donors would be compatible)**
Eurotransplant	**PRA CDC >85%**
UNOS	**PRA CDC >80%**

Sensitivity of Antibody Detection Methods





DSA + at Transplant
“Preformed DSA”

≥35%
Acute Antibody Mediated Rejection

Up to 15%
1- year Graft Failure Rate (non-desensitized)

Le facheur et al. Am J Transplant 2008;8:324

Dunn et al. Am J Transplant 2011;11:2132

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De novo anti-HLA DSA in the first year And beyond

<5%

Acute Antibody Mediated Rejection



DSA - at Transplant

Smith et al. Am J Transplant 2011;11:312

Everly et al. Am J Transplant 2012; In submission

Dunn et al. Am J Transplant 2011;11:2132

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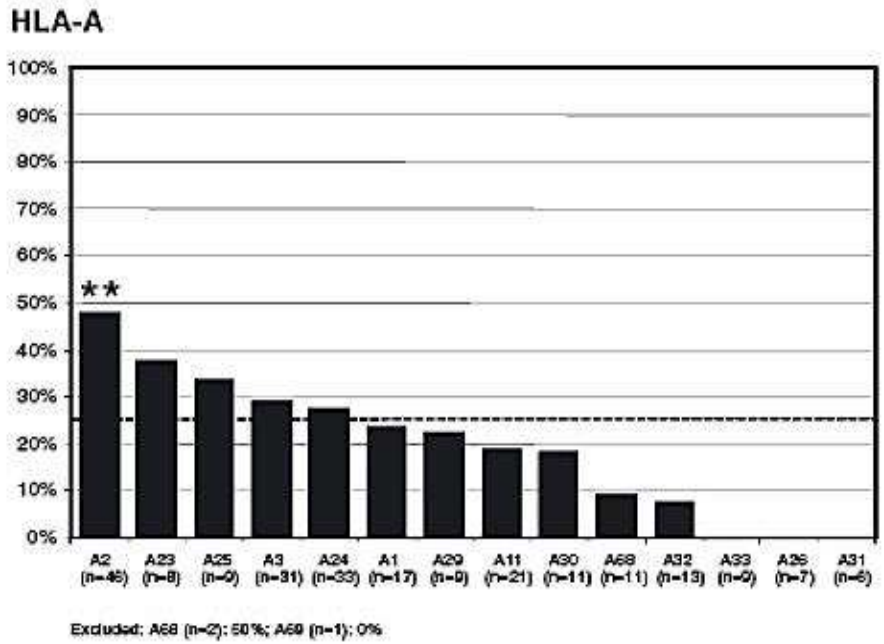
Causes of HLA sensitization

- Transfusion
- Pregnancy
- Transplantation
- Transplant nephrectomy
- Immunosuppression withdrawal

Pregnancies

SAB tests for HLA antibodies 1-4 days after giving birth.

Women with pregnancies from the same male.



HLA sensitization:

- After 1st pregnancy: 33%
- After 2nd pregnancy : 62%
- After 3rd pregnancy : 75%

Against (frequency order):

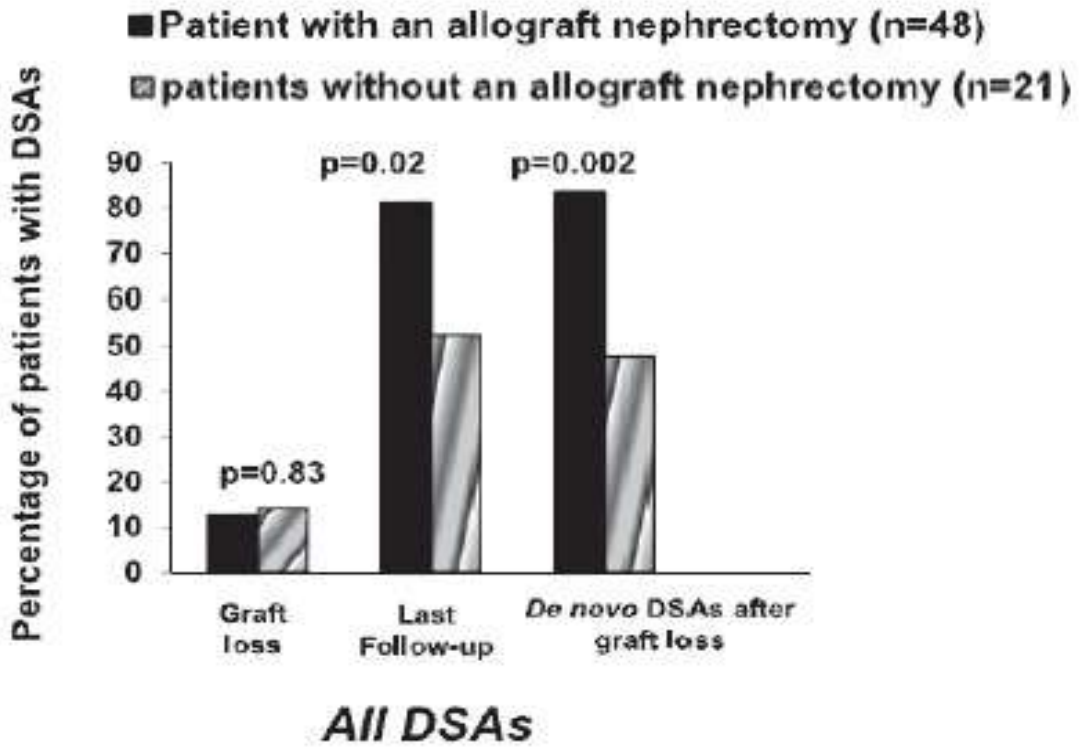
- HLA B, A, DR y C

American Journal of Transplantation 2013; 13: 746-753

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Nephrectomy increases HLA sensitization

Donor-specific antibodies after ceasing IS,
with vs. without allograft nephrectomy



Del Bello A. Clin J Am SocNephrol.2012

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But is it immunosuppression withdrawal?

Independent of nephrectomy, weaning IS leads to late sensitization after kidney transplant failure

	Multivariate logistic regression analyses for PRA ≥80% at 6-24 months after tx failure		
Variables	Odds Ratio	95% CI	*P*
Age at failure (per year)	0.965	0.928–1.004	0.078
Female			
African American	3.110	1.011–9.567	0.048
HLA mismatch	1.803	1.257–2.587	0.001
Living donor			
Allograft survival (per month)	1.000	0.992–1.008	NS
KP transplantation	0.299	0.050–1.789	NS
Weaned immunosuppression	14.342	2.334–88.144	0.004
Transplant nephrectomy	1.893	0.594–6.036	NS

[a] r^2=0.401.

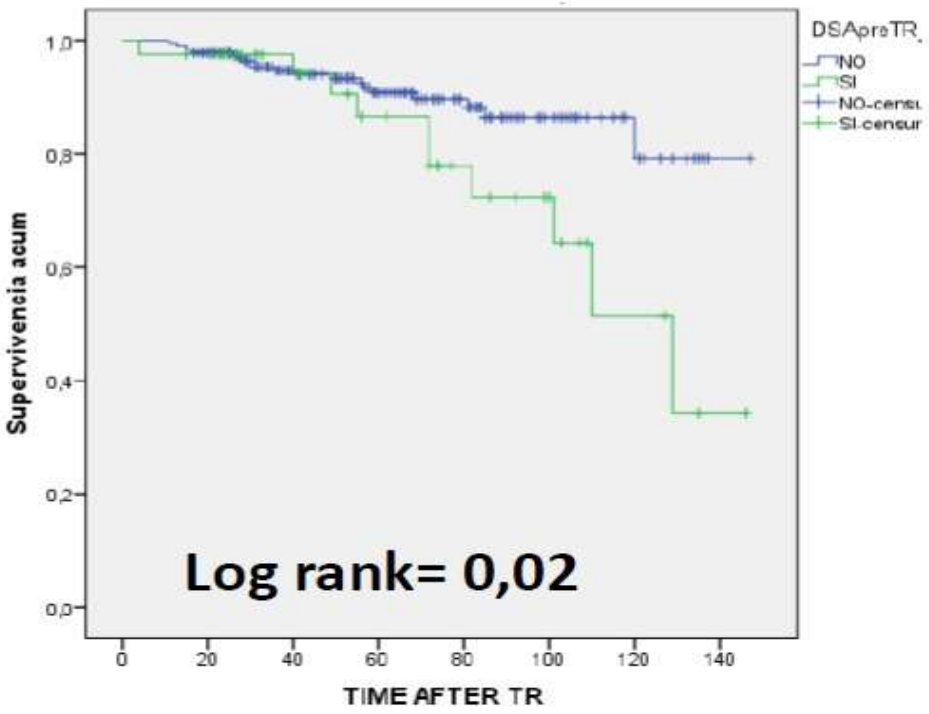
Augustine J.C. et al. Transplantation.2012 Oct 15;94(7):738-43

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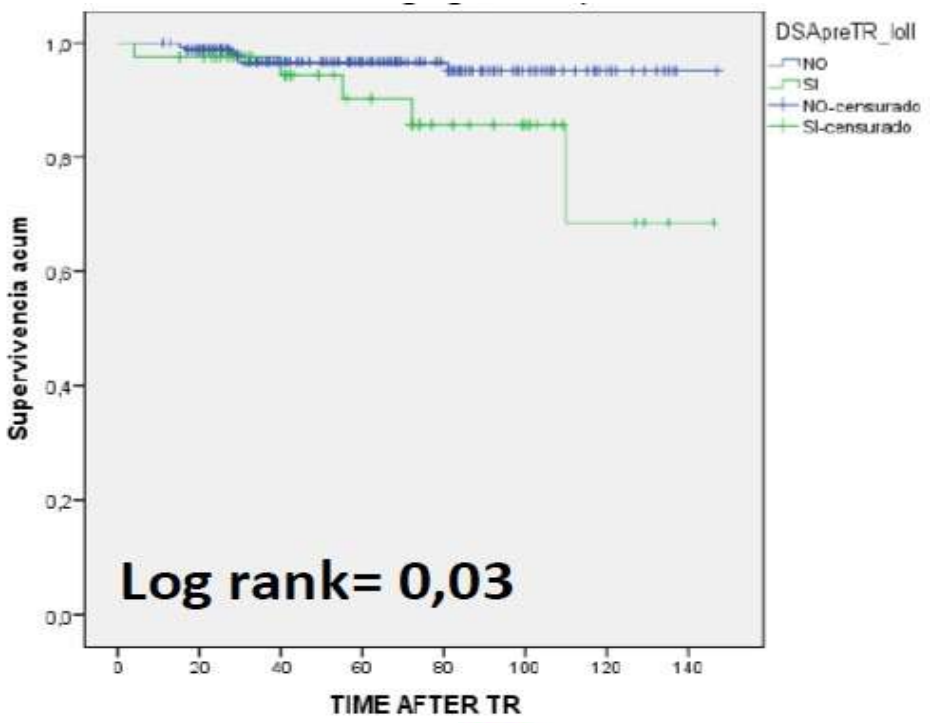
Pretransplant DSA& graft survival

Follow-up 69 months (3-397 months)

(uncensored)



(censoring death with functioning grafts)



Crespo et al. ESOT 2013

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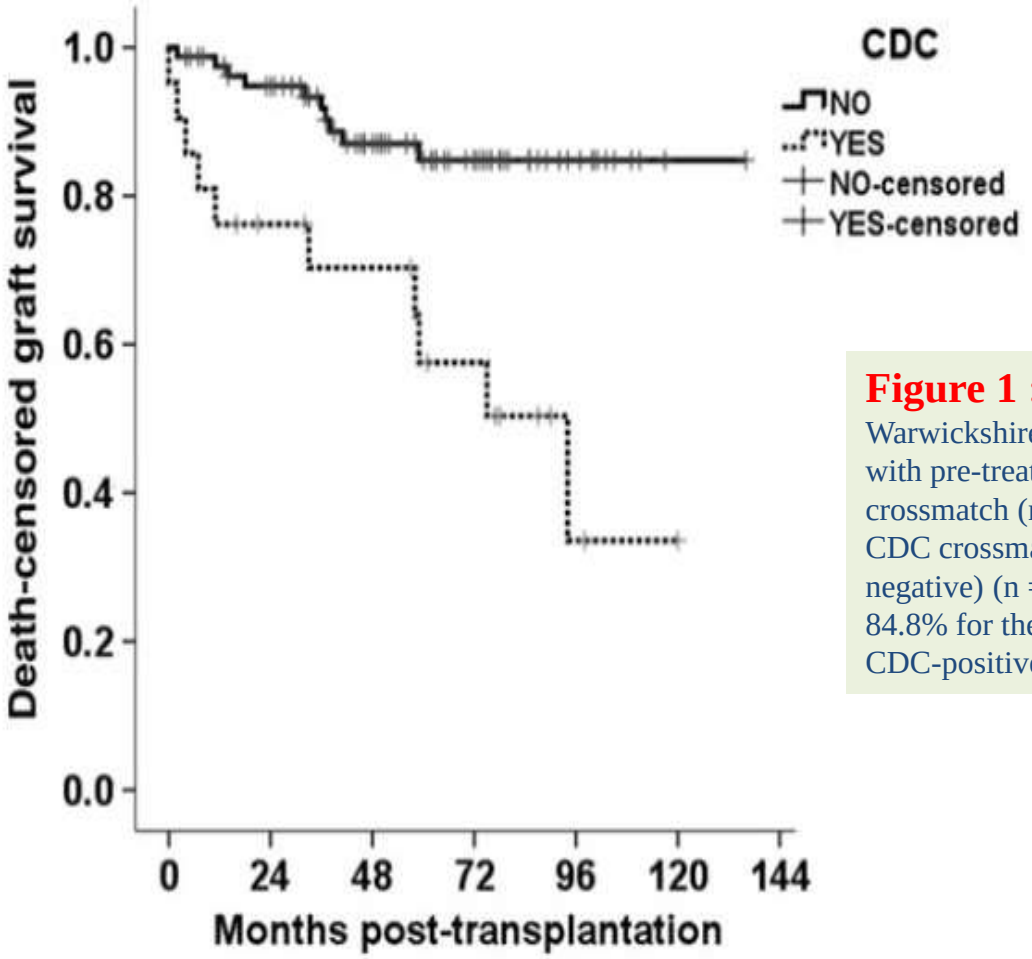


Figure 1 : Graft survival at University Hospitals Coventry and Warwickshire in HLA antibody incompatible transplantation, for patients with pre-treatment complement dependent cytotoxic (CDC) positive crossmatch (n = 21) compared with those with DSA but with a negative CDC crossmatch (includes flow cytometric crossmatch positive and negative) (n = 91). Five- and 10-year death-censored graft survivals were 84.8% for the CDC-negative group, 57.5 and 33.6% respectively for the CDC-positive group.

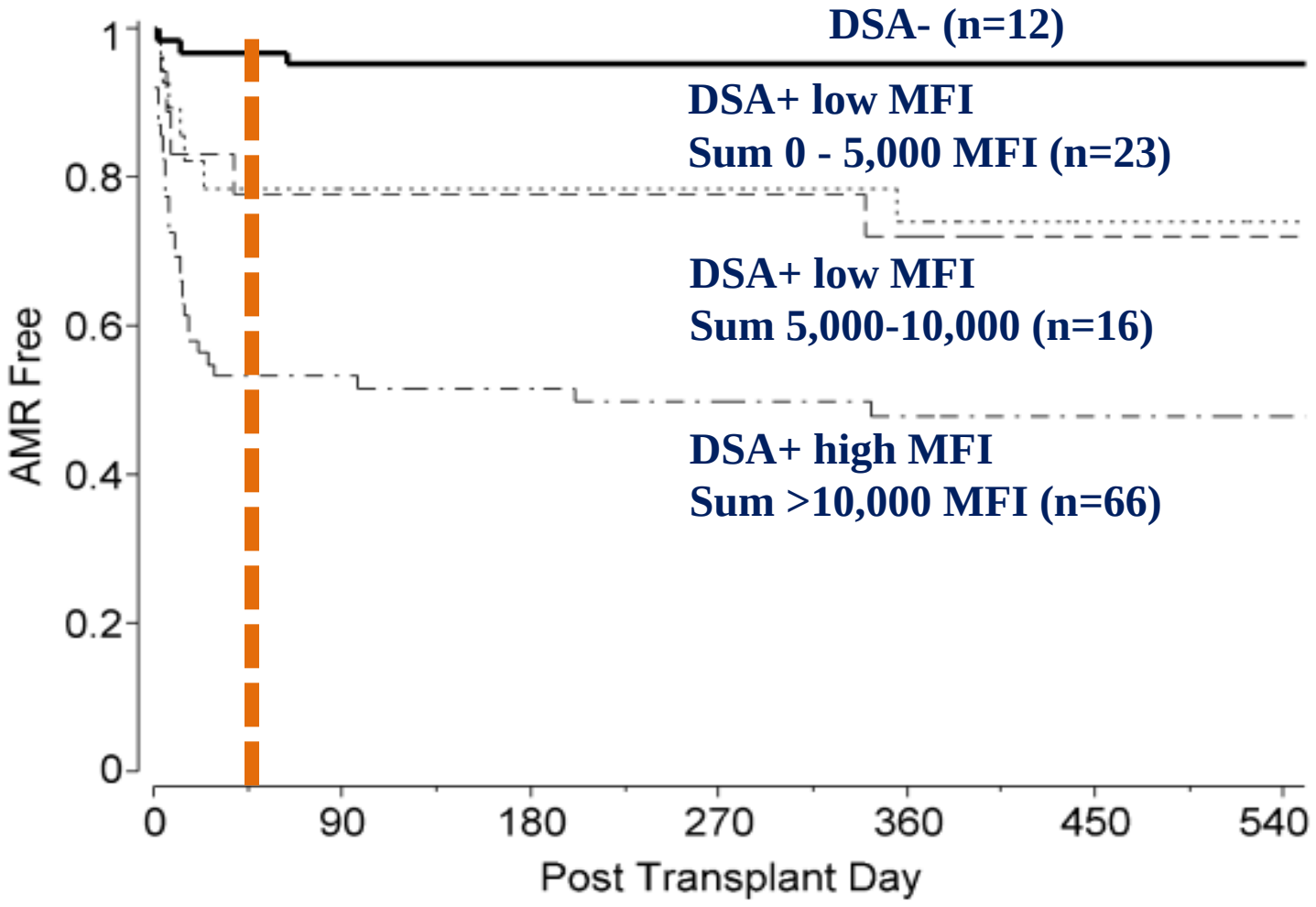
R.M. Higgins et al. Nephrol Dial Transplant (2014) 0: 1–7

Table 1. The clinical relevance of DSA varies amongst different studies

Reference	*N*	DSA+ (*n*)	Type	Donor	Selection	AMR (C4d+)	Graft survival
Gibney *et al.* [11]	136	20	AHG-XM	DD/LD	No		↓
Patel *et al.* [12]	60	20	FCXM	LD	No	↑	↔
Gupta *et al.* [13]	121	16	CDCXM	DD	No		↓
Berg Loonen *et al.* [14]	34	13	CDCXM	DD	AM		↔
Billen *et al.* [15]	165	32	CDCXM	DD/LD	No		↓ I ↔ II
Eng *et al.* [16]	471	27	CDCXM	DD	BXM+	↑	↓
Vlad *et al.* [17]	325	27	CDCXM	DD	No	↑	↔
Amico *et al.* [18]	334	67	CDCXM	DD/LD	No	↑	↓
Wahrmann *et al.* [19]	338	39	CDCXM	DD/LD	No	↑	↓
Aubert *et al.* [20]	114	11	CDCXM	??	No	↔	↔
Phelan *et al.* [21]	64	12	CDCXM (AHG)	LD	No	↔	↔
Morris *et al.* [22]	149	15	CDC/FCXM	LD	No		↔
Lefaucheur *et al.* [23]	402	76	CDCXM	DD	No	↑	↓
Riethmüller *et al.* [24]	37	20	CDCXM	LD	Sens	↑ I ↔ II	↓
Bartel *et al.* [25]	68	51	CDCXM	DD	IA	↔	↔
Couzi *et al.* [26]	45	34	CDC/FCXM	DD	FCXM+	↑	↓
Couzi *et al.* [26]	45	11	CDC/FCXM	DD	FCXM–	↔	↔
Willicombe *et al.* [27]	480	45	CDC/FCXM	DD/LD	AL	↑	↓
Caro-Oleas *et al.* [28]	892	50	CDC	DD	No	↑	↓

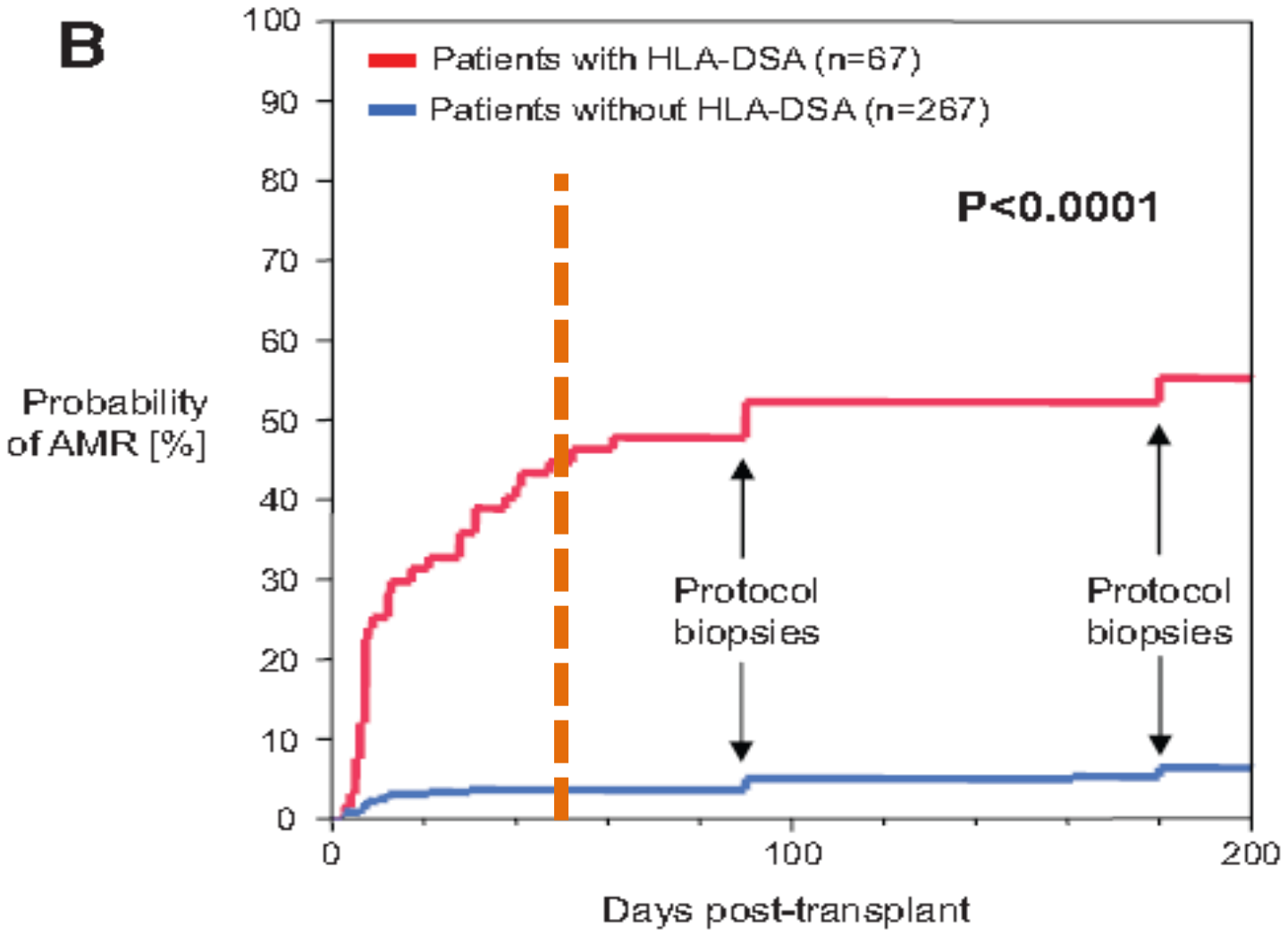
AL, Induction with alemtuzumab; AHG-XM, complement dependent cytotoxicity crossmatch in the presence of anti-human-immunoglobulin; AMR antibody-mediated rejection; BXM, B cell crossmatch; CDC, complement-dependent cytotoxicity; CDCXM, crossmatch in complement dependent cytotoxicity; DD, deceased donor; DSA, donor specific HLA antibodies; FCXM, flow cytometric crossmatch; IA, immunoadsorption; LD, living donor.
↔ No difference.
↑ or ↓ Increased or decreased.

Roelen et al.2012



Gloor et al. Am J Transplant 2010;10:582

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Amico et al. Transplantation 2009;87:1681

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Median Time

to Acute Antibody Mediated Rejection

7.5 days	16 days	16.5 days
Gloor et al. (n=49) Am. J. Tr,2010	**Lefaucheur et al. (n=21)** Am. J. Tr, 2009	**Dunn et al. (n=46)** Am. J. Tr, 2011

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Transplant glomerulopathy risk

47% of XM+ AMR+ Patients

41% of XM+ AMR- Patients

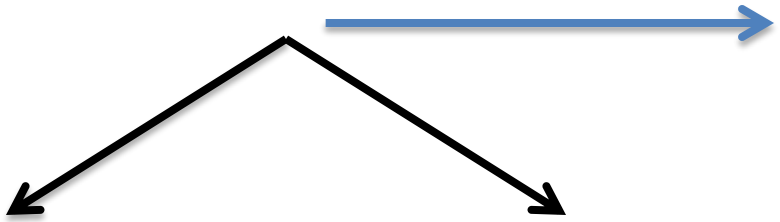
Surveillance of alloantibodies after transplantation identifies the risk of chronic rejection

Pamela M. Kimball[1], Melissa A. Baker[1], Mary B. Wagner[1] and Anne King[1]

[1]Department of Transplant Surgery, Virginia Commonwealth University Health Systems, Richmond, Virginia, USA

69 patients

Flow Positive Crossmatch



21 excluded
no DSA, or not clear group

33

Group 1
Convert to Flow XM -

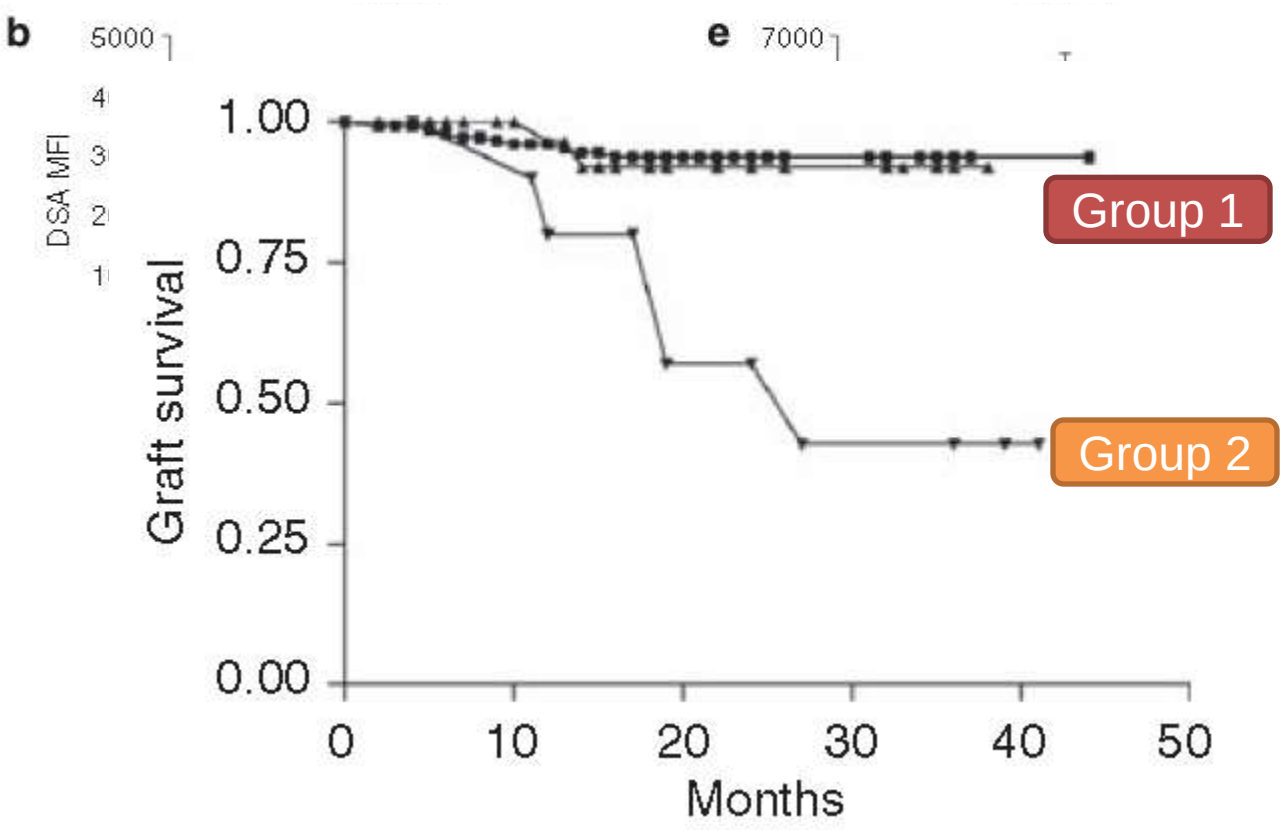
15

Group 2
Remain Flow XM +

Kidney Int 2011;10:582

Group 1

Group 2



Kimball et al. Kidney Int 2011;10:582

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Monitoring Time Points	
Pre-Transplant, Day of Transplant	
1 week	With for cause biopsy
2 weeks	
4 weeks	
8 weeks	
6 months	
12 months	
After 12 months *	

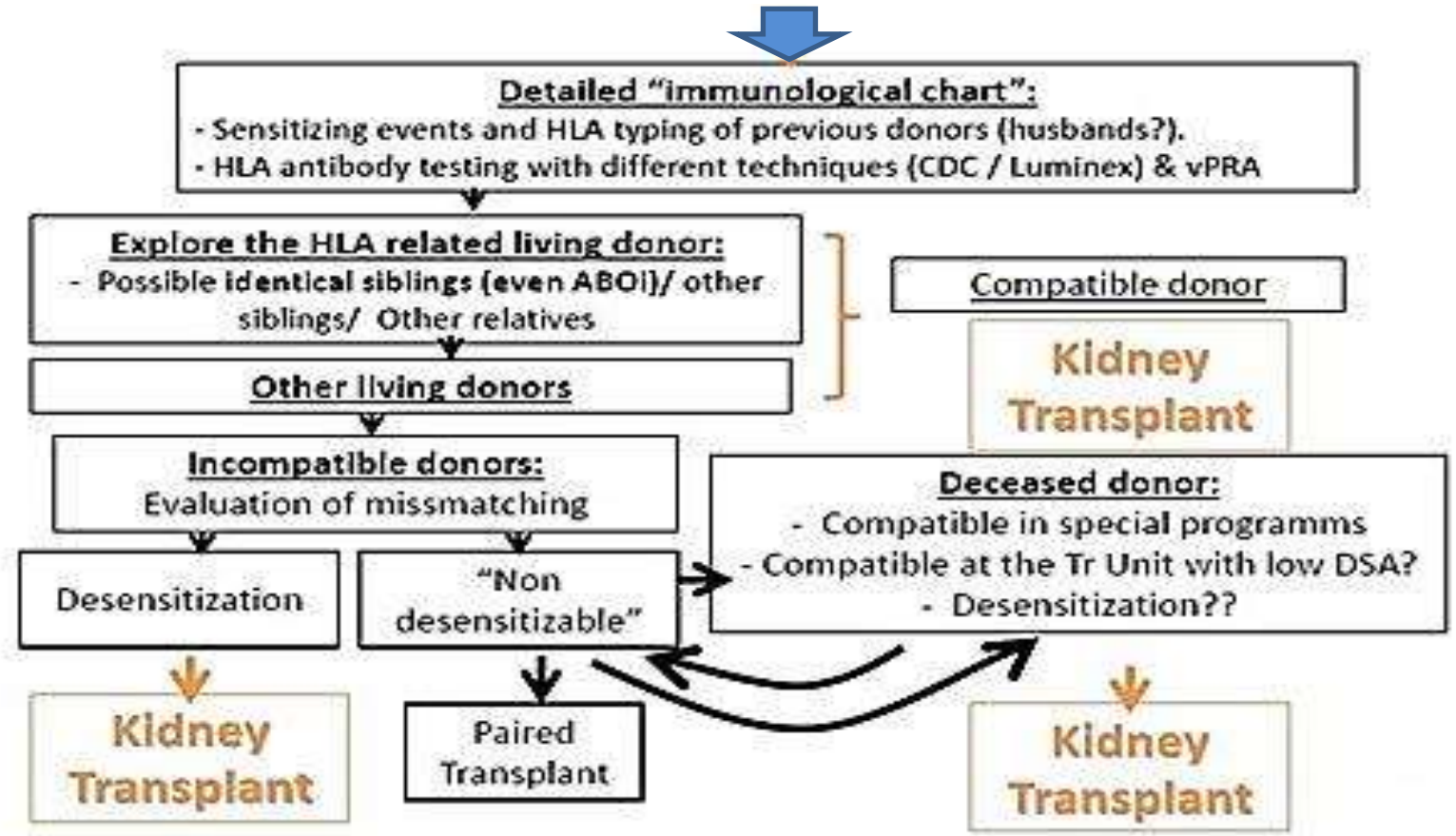
*** If patient's DSA remain persistently positive – treatment may be useful to improve outcomes.**

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How to manage sensitized patients ?

- **Suggested algorithm**
- **Desensitization**
- **Accetable missmatch programme**
- **Paired kidney donation**

Algorithm


Highly sensitized patient with CDC-PRA> 80%?
Detailed "immunological chart":
- Sensitizing events and HLA typing of previous donors (husbands?).
- HLA antibody testing with different techniques (CDC / Luminex) & vPRA
Explore the HLA related living donor:
- Possible identical siblings (even ABOi)/ other siblings/ Other relatives
Other living donors
Compatible donor
Kidney Transplant
Incompatible donors:
Evaluation of missmatching
Deceased donor:
- Compatible in special programms
- Compatible at the Tr Unit with low DSA?
- Desensitization??
Desensitization
"Non desensitizable"
Kidney Transplant
Paired Transplant
Kidney Transplant

Strategies to desensitize before TR

Techniques to eliminate Abs

-PE

-IA

Techniques to "block" Abs

-IVIG

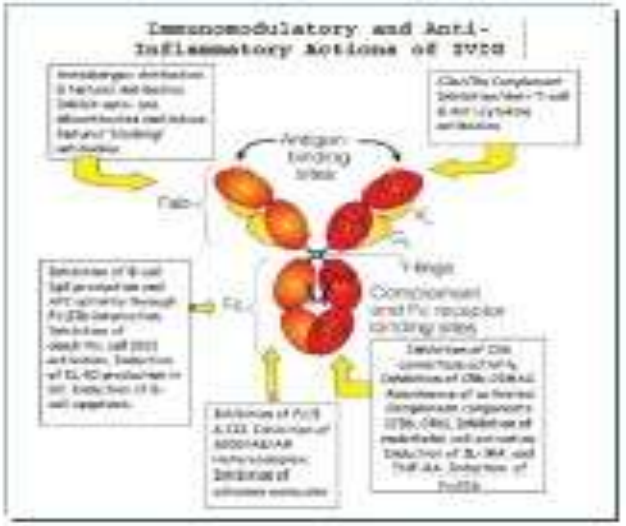


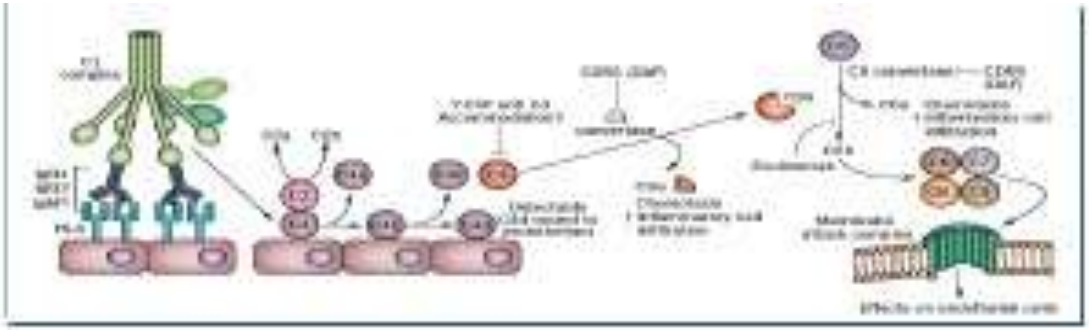
Other strategies ??

-Rituximab

-Bortezomib?

-Eculizumab





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Desensitization protocols

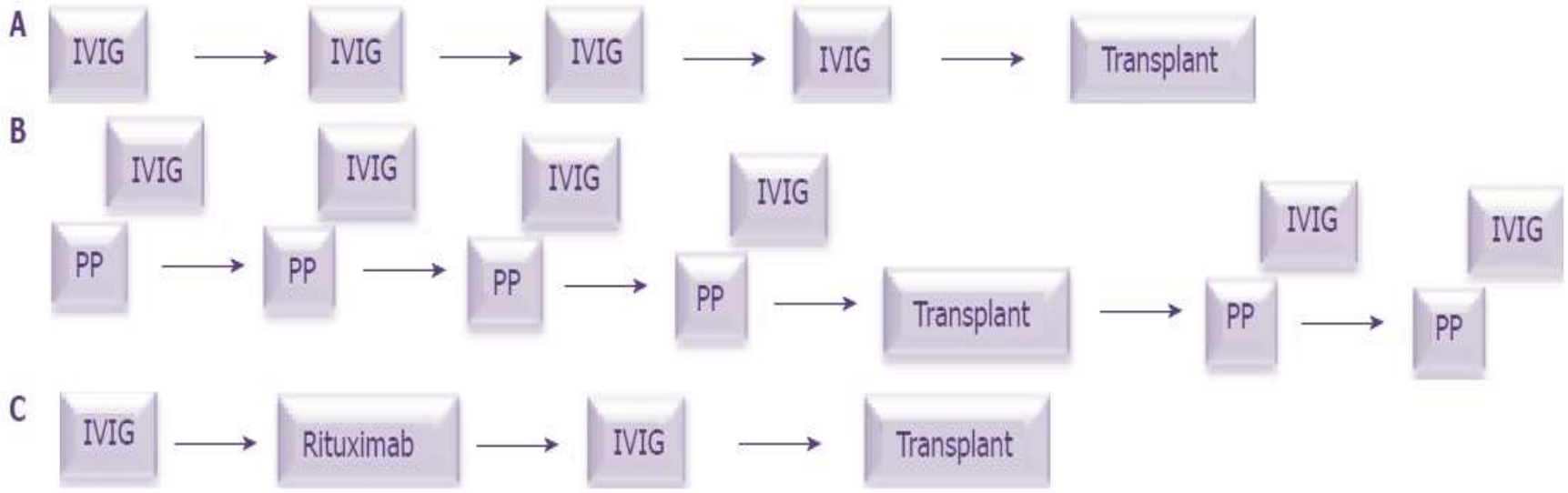
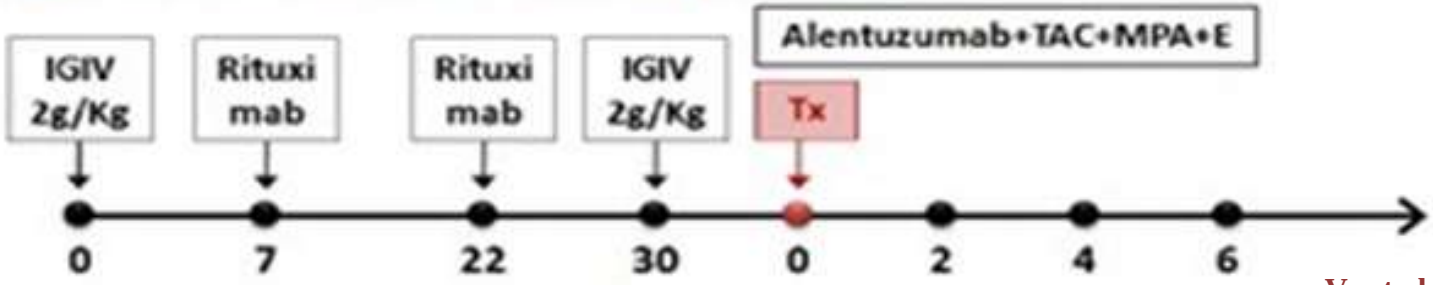


Figure1. A: The NIH Ig02 trial administered intravenous immunoglobulin (IVIG) in four monthly doses for patients awaiting a living or deceased donor transplant. This was followed by a living or deceased donor transplant once an acceptable crossmatch was achieved; B: Johns Hopkins University used a combination plasmapheresis (PP) with low-dose cytomegalovirus immune globulin following each PP session. The number and frequency of the PP sessions is dependent on the donor specific antibody titer. A living donor transplant occurs when an acceptable crossmatch is achieved. Additional sessions of PP/IVIG are administered after transplant; C: A modified protocol combining IVIG and rituximab was developed at Cedars-Sinai Medical Center. Two doses of IVIG are administered one month apart with one dose of rituximab given in between. A deceased or living donor transplant then takes place when an acceptable crossmatch is obtained.

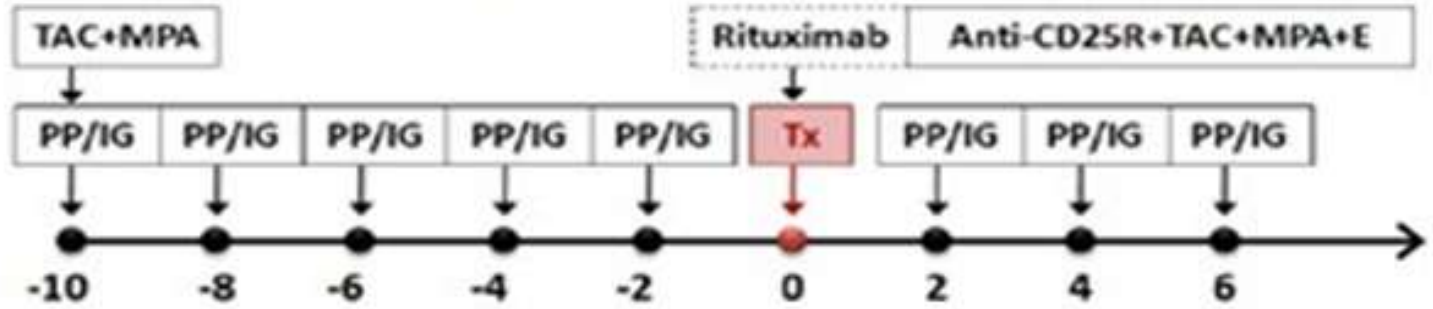
Strategies to desensitize with a living donor

High dose iv immunoglobulin



Vo et al . NEJM 2008

Plasmaexchange/Immunoadsortion + low dose IGIV



Montgomery . AJT 2010

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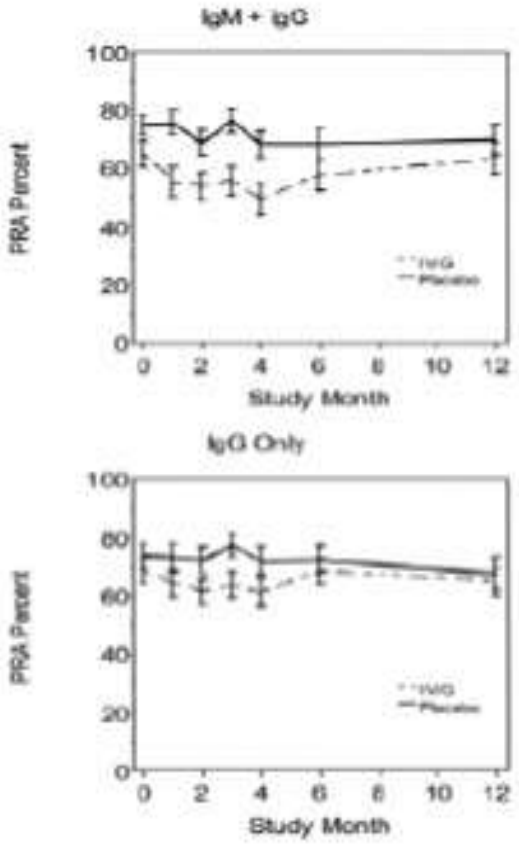
Desensitization in the waiting list with IVIG

NIHIGO2 study Am J Tr 2005

Multicenter, randomized, double blind

End-points	IVIg N= 48 (%)	Placebo N= 50 (%)
Transplant	17 (35%)	10(17%)
Waiting Time *	4.8 ys	10.3 ys
Acute rejection	9(35%)	1 (10%)‡
Graft loss	4(25%)	3(38%)
2 years graft survival	80%	75%
Serum Creat (mg /dl)	1.68±0.2	1.28±0.13

*** p=0.02**

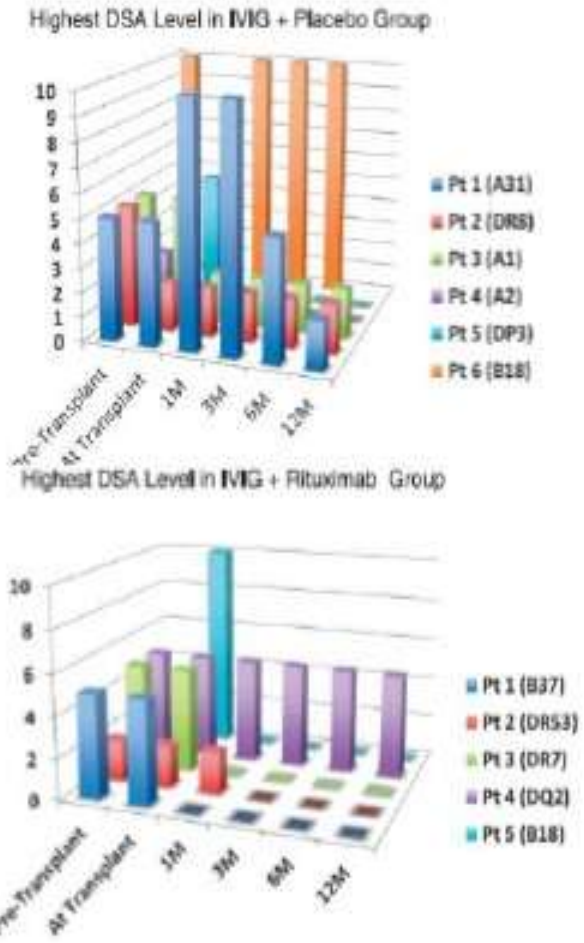


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Rituximab Combined (Waiting List)

2014 Unicenter randomized trial NON blinded, n=13

	IvIG+ placebo n=7	IvIG+ Rituxima, n=6
Time to TR (mean)	7 months	6 months
AMR	43%	**0%** ***p=0.06**
ACR	14%	17%
Graft Loss	29%	0%
Death with fn graft	0	17%
1 year creat	**2+0.6**	**1.1+0.36** ***p=0.046**



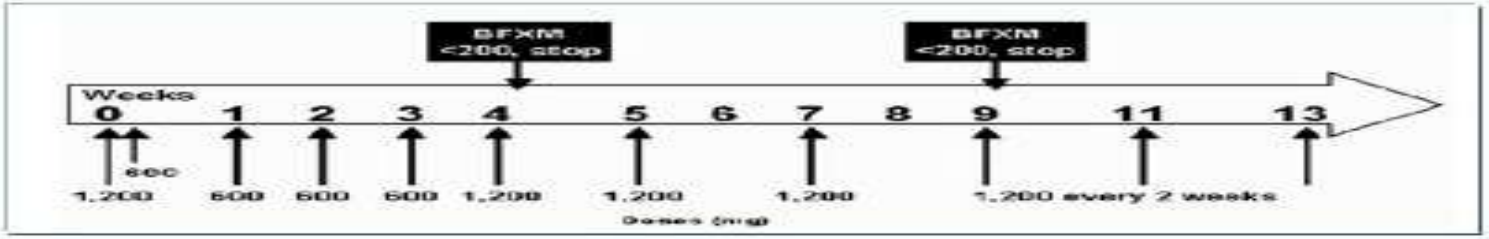
Transplantation . Volume 98, Nuvember 3, August 15, 2014

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Other strategies

Complement Blocking (Eculizumab):

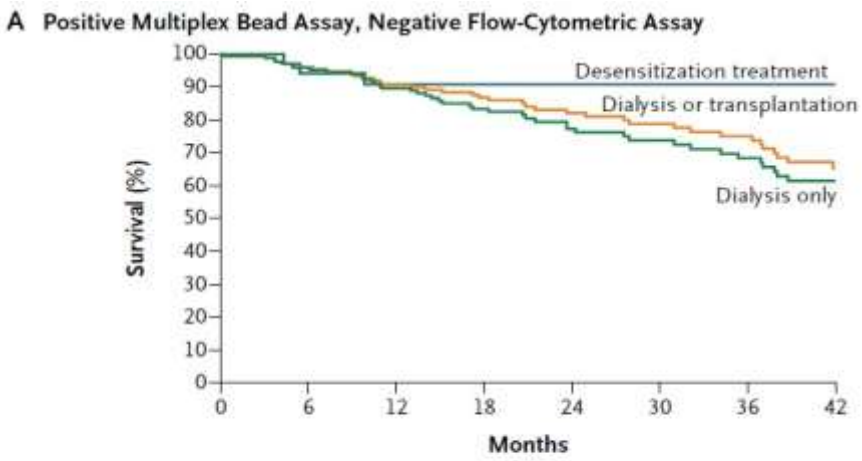
Living TR with eculizumab vs historical



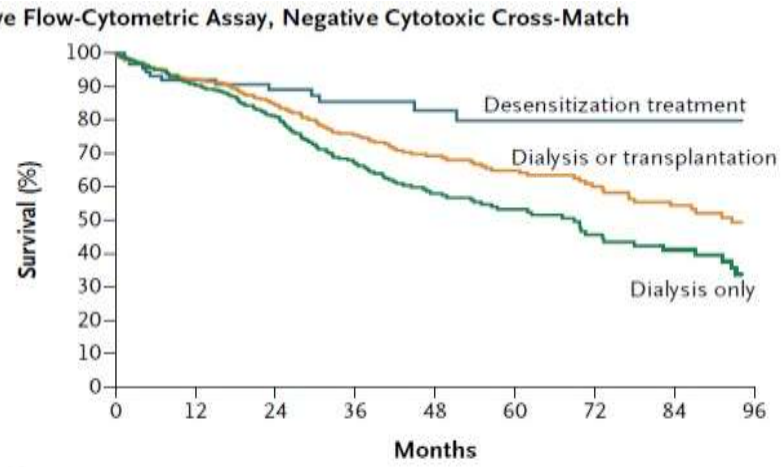
Category	Eculizumab group (n = 26)	Control group (n = 51)	p-Value
Follow-up (mean months ± SD, range)	11.8 ± 6.3 (3.0–27.5)	48.8 ± 14.1 (7.8–69.8)	
Graft survival at 1 year (n, %)	16/16 (100%)	49/51 (96%)	1.00
Antibody-mediated rejection ≤ 3months (n, %)	2 (7.7%)	21 (41%)	0.0031
Patients developing high DSA levels ≤ 3 months[1]	13 (50%)	22 (43%)	0.63
High DSA biopsies C4d+ (n, %)	13 (100%)	20 (91%)	0.52
High DSA and C4d+ biopsies showing AMR (n, %)	2 (15%)	20 (100%)	<0.0001
Cellular rejection ≤3 months (n, %)	1 (6.2%)	1 (2%)	0.42
Plasma exchange posttransplant			
Patients receiving PE (n, %)	3 (12%)	39 (76%)	<0.0001
Number of PE treatments (mean ± SD)	0.35 ± 1.1	7.9 ± 7.5	<0.0001

Of 16 Living-donor TR with1 year follow-up: 8 stop <8 weeks& 2 continue at 1 year.

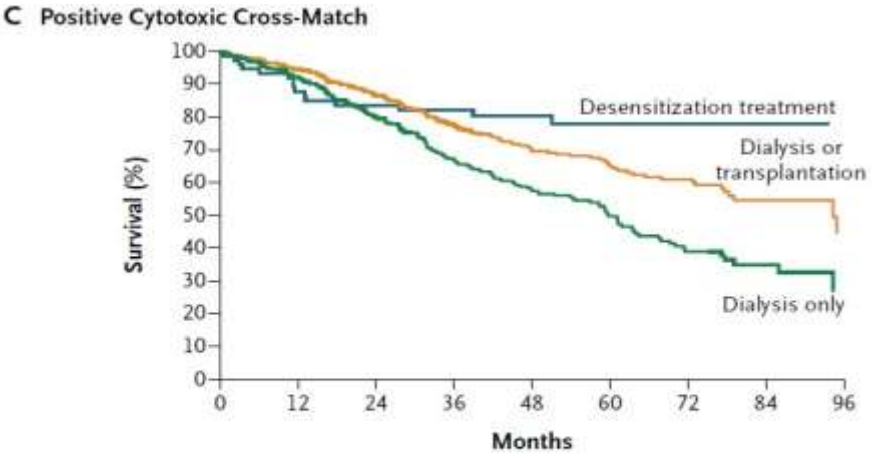
American Journal of Transplantation 2011; 11: 2405–2413



No. at Risk								
Desensitization treatment	38	30	26	23	18	15	14	9
Dual therapy	187	152	129	110	84	66	59	31
Dialysis only	182	147	123	101	74	57	50	27



No. at Risk									
Desensitization treatment	88	71	58	39	27	23	17	16	10
Dual therapy	425	346	276	176	114	94	67	56	27
Dialysis only	420	335	260	154	88	68	43	35	12



No. at Risk									
Desensitization treatment	74	63	57	48	38	31	23	11	3
Dual therapy	365	330	281	221	163	115	79	35	9
Dialysis only	360	317	252	184	132	81	48	19	5

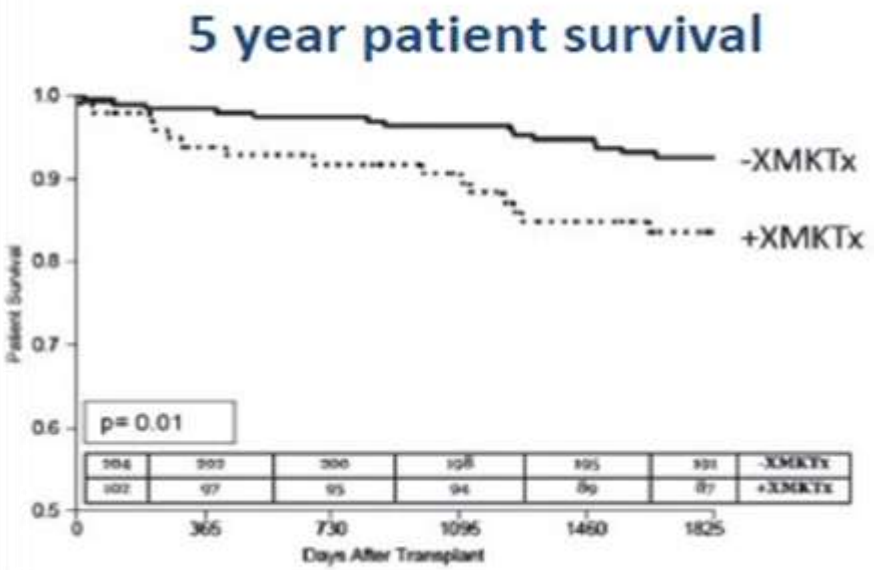
Figure 2. Survival Benefit of Desensitization According to Strength of Cross-Matching for Donor-Specific Anti-HLA Antibody.

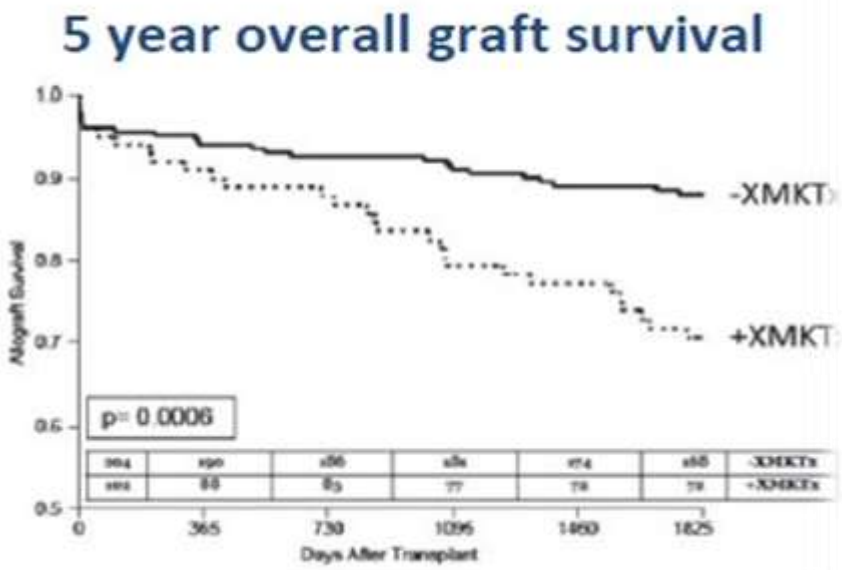
Kaplan–Meier survival estimates are shown for kidney recipients in the treatment group, as compared with the two matched control groups of patients on a kidney waiting list who continued to receive dialysis (dialysis-only group) or who either continued to undergo dialysis or underwent HLA-compatible transplantation (dialysis-or-transplantation, or dual therapy, group), according to whether donor-specific anti-HLA antibody was detected on the multiplex bead assay (Panel A), flow-cytometric assay (Panel B), or positive complement-dependent cytotoxicity assay (Panel C).

N Engl J Med. 2011 Jul 28;365 (4):318-26

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Long-term Results Inferior To TR Without DSA





- Comparison between 102 (+)XMKTx vs 204 (-)XMKTx matched for age and sex
- 5-year death-censored GS was lower in the +XMKTx (70.7% vs. 88.0%, p < 0.01)
- Graft survival was higher with DSA class I only (vs class II or class I+II) (85.3% vs. 62.6%, p = 0.05) and was similar to -XMKTx (85.3 vs. 88.0%, p = 0.64).

BentallA et al . American Journal of Transplantation 2013; 13: 76–85

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Finding A compatible donor: special programs

Recipient of KTR
PRA>80-95%



Programms sharing donors:

-Acceptable mismatch (ET) based on identifying **acceptable MM**

-**USA** based on **virtual XM**

-Spain based on **virtual XM**

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Acceptable mismatch program

- **Basis:** **definition of those HLA antigens toward which the patient did never form antibodies and use this knowledge for donor selection.**
- **Aim:** **Prediction of a negative CDC crossmatch.**
- **Method:** **once a donor becomes available with an HLA type, which is compatible with the combination of a patients own HLA and one or more acceptable missmatches, the kidney is madatorily shipped to this recipient.**

The AM program (ET-III): results

Enhanced Kidney Allocation to Highly Sensitized Patients by the Acceptable Mismatch Program

Frans H. J. Claas,[1] Axel Rahmel,[2] and Ilias I. N. Doxiadis[1]

Desde 1997

TABLE 1. Comparison of the degree of HLA matching between the AM and the ET cohort

	No. HLA Mismatches					
	AM (%)			Total ET (%)		
	0	1	2	0	1	2
HLA-A	50	38.7	11.3	38.1	49.5	12.4
HLA-B	34.7	50	15.3	30.9	57.9	11.2
HLA-DR	67.3	32	0.7	56.3	42.4	1.3

HLA, human leukocyte antigen; ET, Eurotransplant; AM, acceptable mismatch.

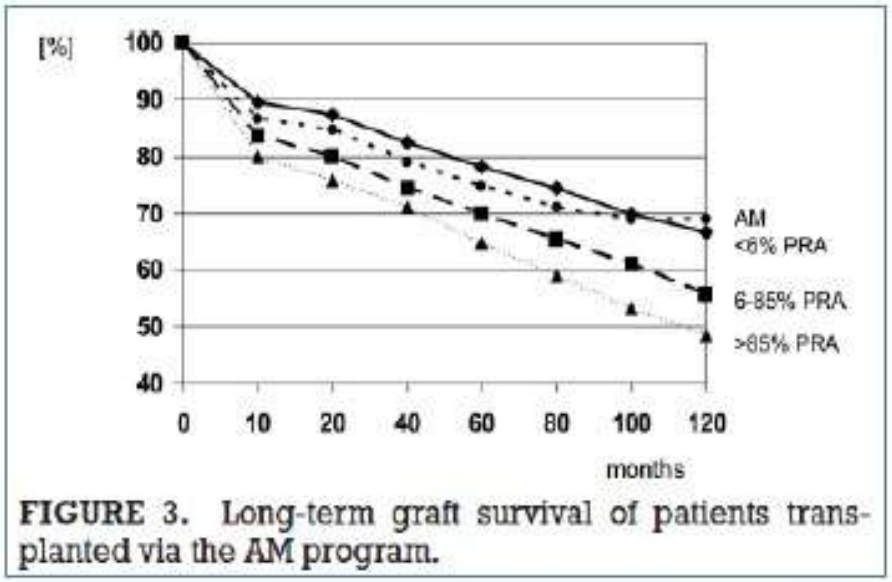


FIGURE 3. Long-term graft survival of patients transplanted via the AM program.

Transplantation , Volume .88, Nuvembert4, August 27, 2009

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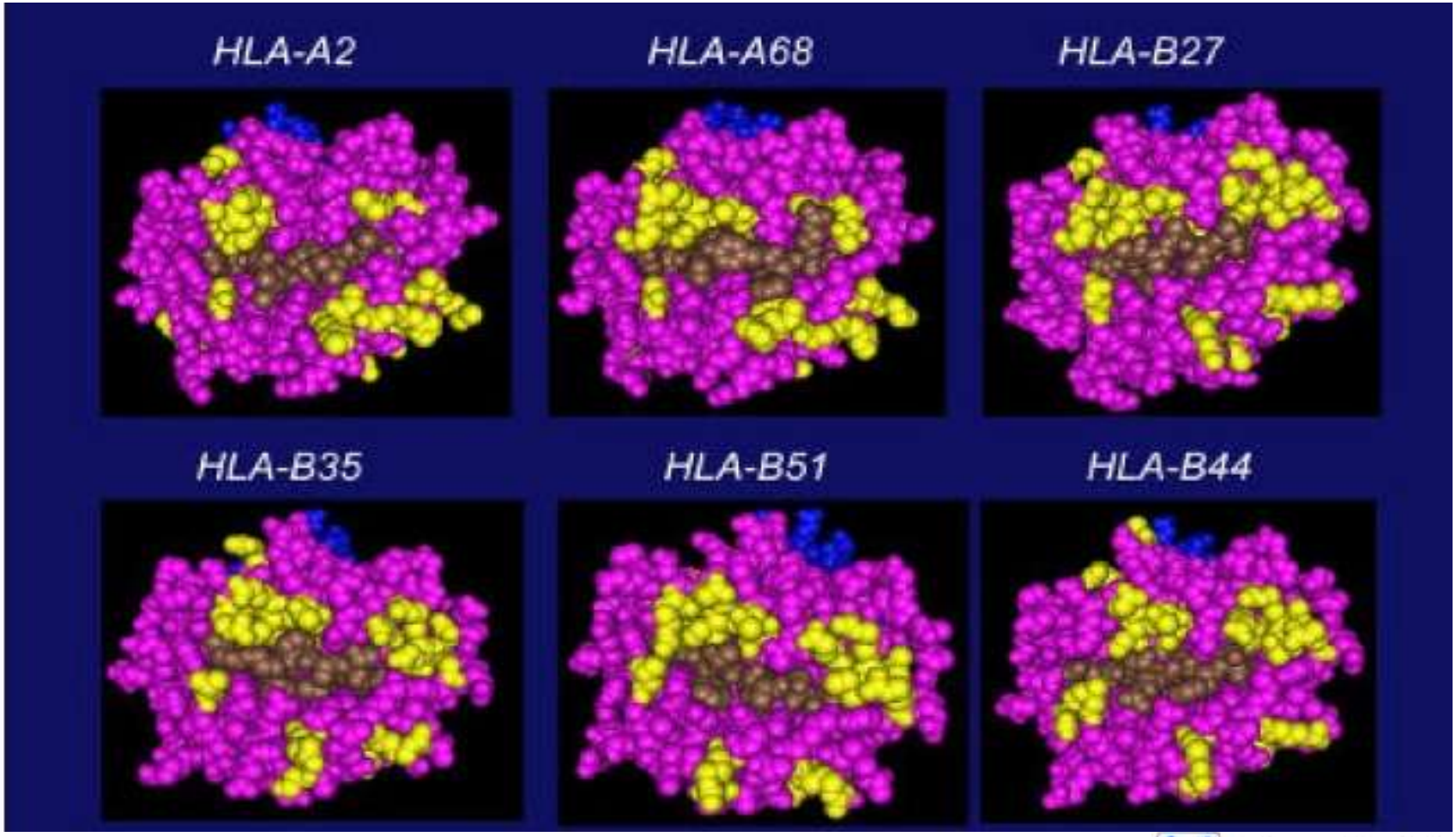
Toward alternative matching strategies

- **The number of HLA antigens is increasing enoromusly making HLA antigen matching more and more difficult.**

- **Aim: to develop an alternative matching algorithm based on the relevant epitopes* without the need for conventional HLA antigen matching.**

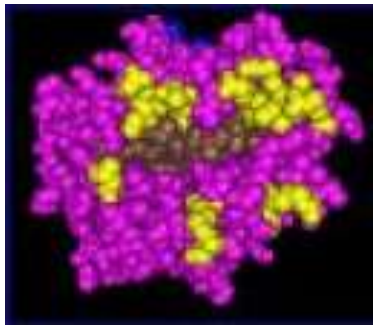
***the more than 5,000 HLA class I allels can be defined on basis of 180 epitopes**

Analysis of the antibody epitopes on HLA molecules enables the prediction of their immunogenicity

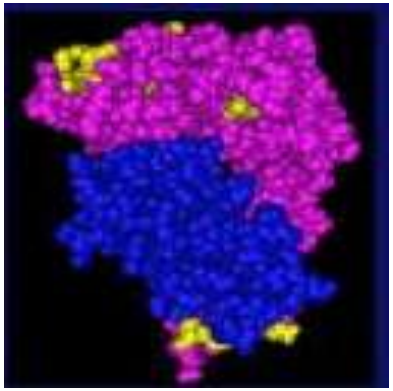


How to predict the immunogenicity of an HLA alloantigen

1. Antigen has many potentially immunogenic epitopes but some epitopes are shared with patients' own HLA molecules.

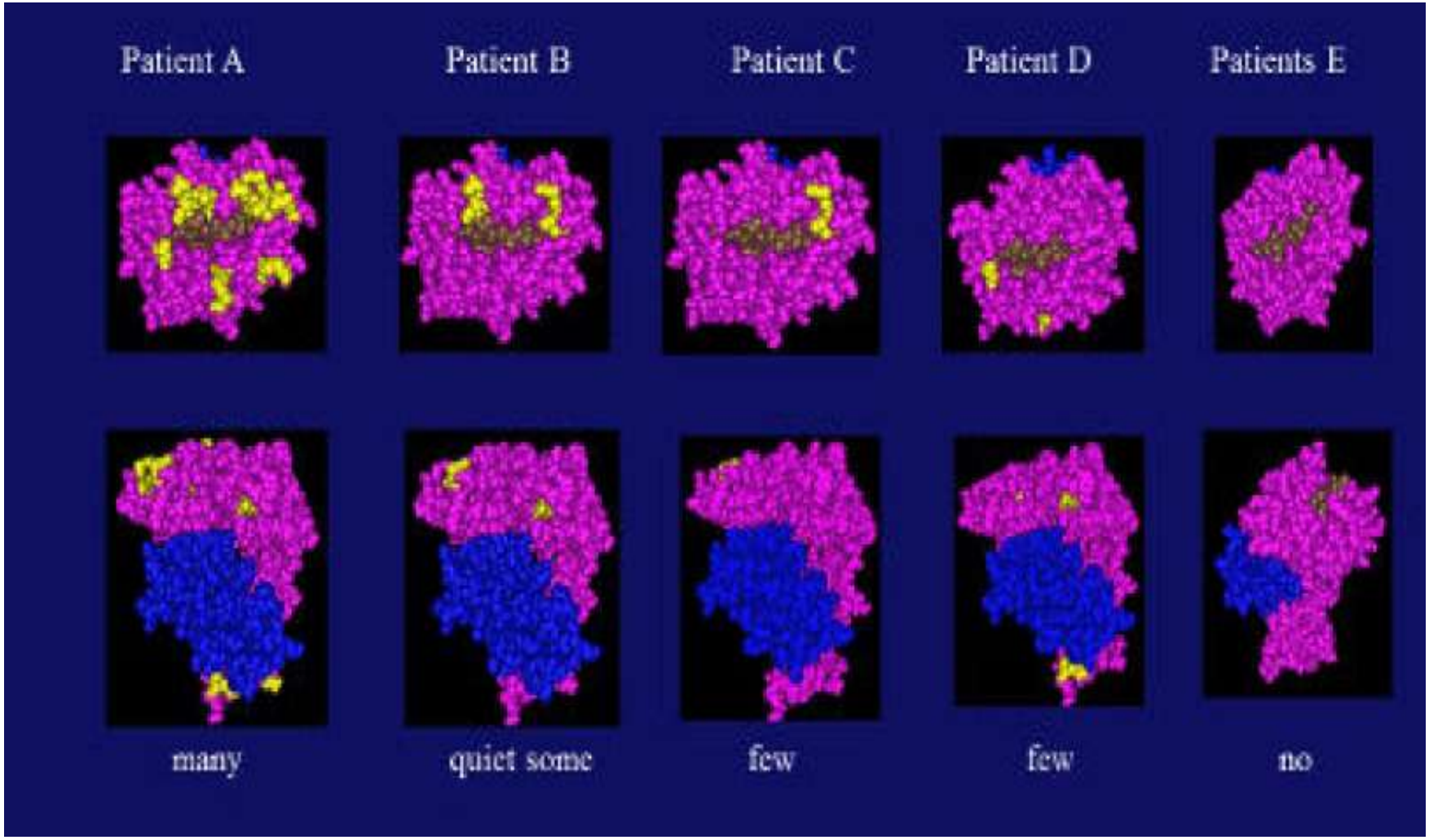


2. Patient will not make antibodies to epitopes present on the own HLA antigens and therefore:

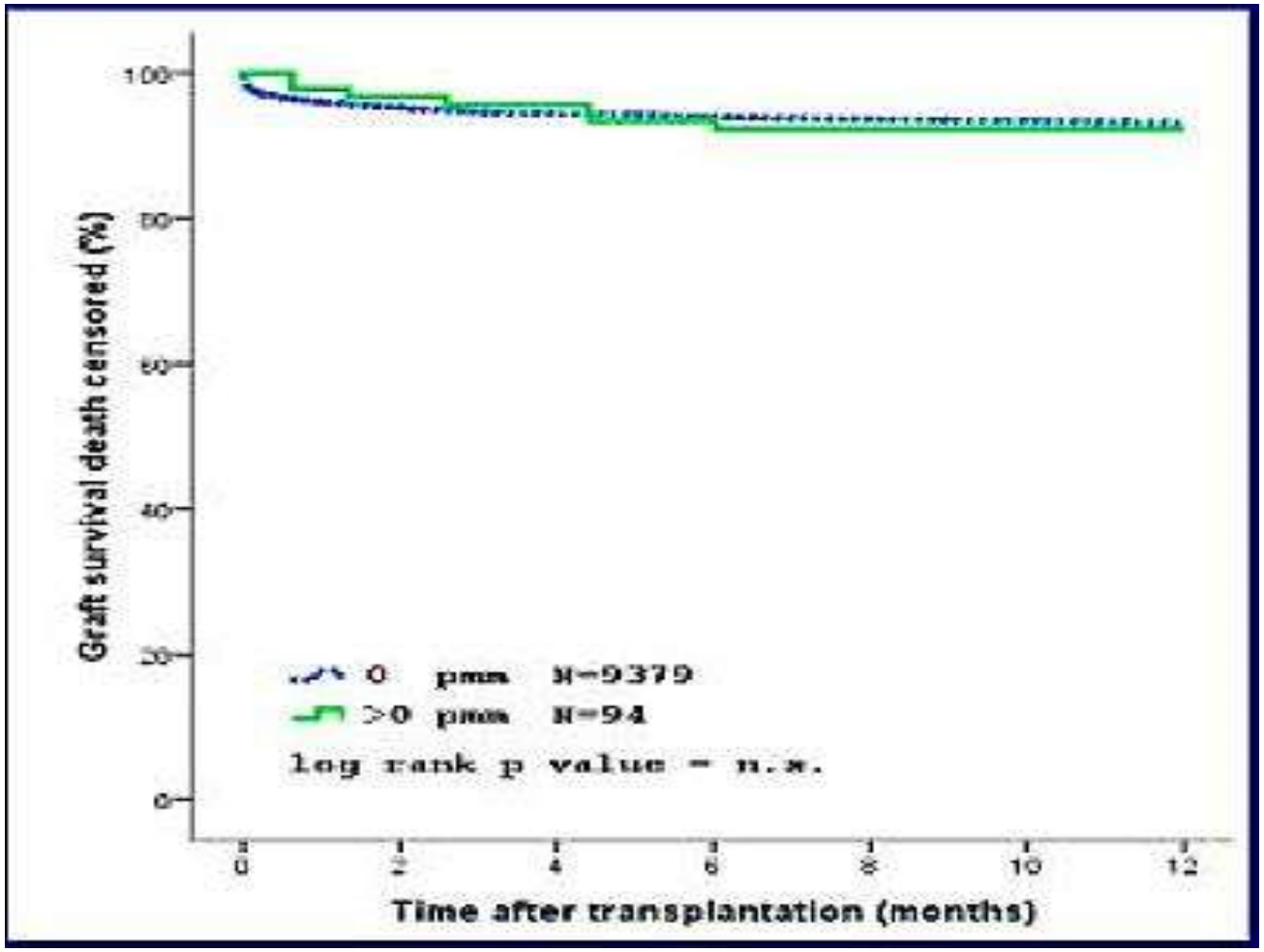


3. Polymorphism of an HLA mismatch should be considered in the context of the HLA type of the potential antibody producer.

Number of foreign epitopes on the same HLA- B51 mismatch for



Graft survival of epitope matched, HLA antigen mismatched grafts is similar to HLA identical transplants



Doxiadis et al, 2012

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Paired Kidney Donation

To match the largest possible number of donor–recipient pairs while minimizing immunologic risk. KPD programmes use sophisticated algorithms to identify suitable matches with simultaneous two-way or more complex multi-way exchanges as well as including non-directed anonymous donors to start a chain of compatible transplantations.

Paired kidney exchange paradigms

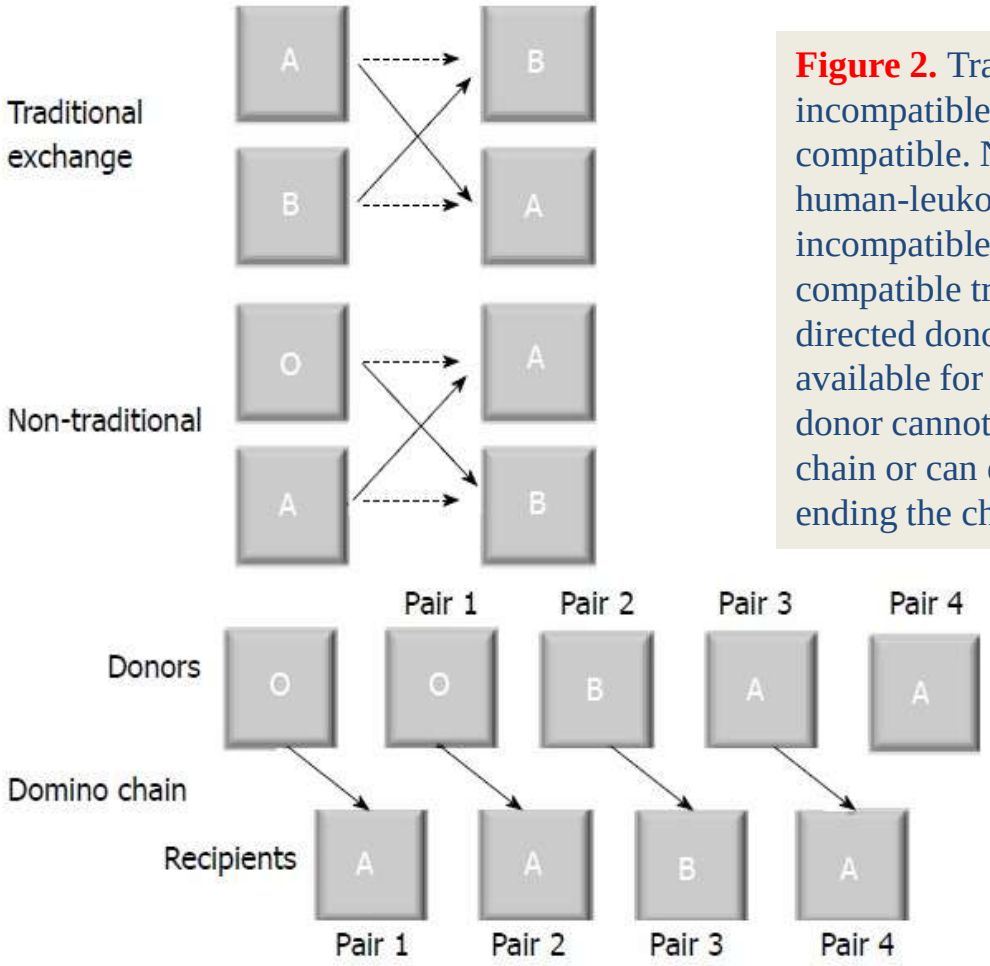


Figure 2. Traditional exchange: This exchange swaps two ABO incompatible pairs so that new donor recipient pairs are now ABO compatible. Non-traditional exchange: This exchange uses one human-leukocyte antigen (HLA) incompatible pair and one ABO incompatible pair and trades for two HLA compatible and ABO compatible transplants. Domino chain: This chain starts with a non-directed donor. The recipient of this kidney then has their donor available for another recipient. This occurs progressively until a donor cannot be matched. This donor can act as a bridge to a new chain or can donate to the deceased donor waiting list, thereby ending the chain.

Post-transplant DSA Monitoring May Predict Antibody-Mediated Rejection in Sensitized Kidney Transplant Recipients

Maha A Mohamed[1], Brenda Muth[1], Vijay Vidyasagar[1], David Foley[2], Luis Fernandez[2], R. Michael Hofmann[1], Josh Mezrich[2], John Pirsch[2], Jon Odorico[2], Tony d'Alessandro[2], Janet Bellingham[2], Jose Torrealba[3], Dixon Kaufman[2], and Arjang Djamali[1, 2]

Departments of Medicine[1], Surgery[2], Pathology and Laboratory Medicine[3], University of Wisconsin, Madison School of Medicine and Public Health, Madison, Wisconsin

116 Flow Negative Crossmatch, but DSA Positive

Table 3. Cox proportional hazard model for predictors of AMR.

	Univariate			Multivariate		
	HR	95% CI	p	HR	95% CI	p
Increase in DSAmax at 1 week	1.0004	1.0002 - 1.0006	0.0001	1.0002	1.0001 - 1.0004	0.03
Peak PRA	1.008	0.99 - 1.01	0.11	-	-	-
Re-transplant	1.3	0.76 - 2.21	0.3	-	-	-
African American	2.8	0.63 - 12.2	0.2	-	-	-
Age	0.96	0.93 - 0.99	0.03	0.98	0.94 - 1.02	0.4
DSAmax at transplant	1.0002	1.000 - 1.0005	0.06	-	-	-
ATG Induction	5.2	2.05 - 13.3	0.0006	4.2	1.26 - 14.14	0.02

Clinical Transplants 2011.

Therapy options for AMR

- The suppression of the T-cell–dependent antibody response (steroids, cyclosporine, tacrolimus, and sirolimus).
- The removal of donor reactive antibody (plasmapheresis).
- The blockade of the residual allo-antibody (IvIg).
- The depletion of naive and memory B cells (rituximab).
- Inactivation of plasma cells (bortezomib).
- The blockade of complement component C5 by monoclonal antibodies (eculizumab).

Kidney International (2012) 81, 628–639

Treatment of Acute AMR:

FDA Workshop

Becker et al.	27	Rituximab 375 mg/m^2 × 1 dose methylprednisolone 500 mg once daily × unspecified duration, rATG 1.5 mg/kg/day × unspecified duration, and with or without apheresis maintenance: calcineurin-inhibitor + mycophenolate mofetil + corticosteroids	Follow-up: 605 days reversibility: not reported graft survival: 85% *(excluding deaths)* creatinine: 0.95 mg/dL
Faguer et al.	8	PP once daily × 9 days (range 2–17 sessions), rituximab 375 mg/m^2 once weekly × 4 weeks,[c] and methylprednisolone 10 mg/kg/day × 3 doses,[d] maintenance: tacrolimus + mycophenolate mofetil + corticosteroids	Reversibility: 75% follow-up: 10 months graft survival: 75% creatinine: 1.7 mg/dL
Böhmig et al.	10 (group A: 5, group B: 5)	Group A: tacrolimus + antilymphocyte antibody + immunoadsorption (9–14 sessions)[e] *compared to* group B: tacrolimus + antilymphocyte antibody[f]	Follow-up: 24 months reversibility: group A = 100%, group B = 20% graft survival: group A = 80%, group B = 20% creatinine: group A = 2 mg/dL, group B = 1.6 mg/dL
Everly et al.	6	PP unspecified regimen, rituximab 200–500 mg/m^2 × 1 dose, and bortezomib 1.3–1.5 mg/m^2 × 4 doses maintenance : tacrolimus + mycophenolate mofetil + corticosteroids	Reversibility: 67%
Walsh et al.	2	PP on days 1, 4, 8, 11, 14, 16 and 18, rituximab 375 mg/m^2 on day 1, bortezomib 1.3 mg/m^2 on days 1, 4, 8 and 11, methylprednisolone 100 mg on days 1 and 4 and 50 mg on days 8 and 11 maintenance: tacrolimus + mycophenolate mofetil + corticosteroids	Reversibility: 100%

American Journal of Transplantation 2011; 11: 896–906

Treatment of acute AMR:
FDA workshop

Lefaucheur et al.	24 (group A: 12 patients enrolled between January 2000 and December 2003, group B: 12 patients enrolled between January 2004 and December 2005)	Group A: IVIG 2 g/kg x 4 doses, PP unspecified, rituximab 3.75 mg/m^2 x 2 doses and methylprednisolone 500 mg x 3 doses *compared to* group B: PP once daily x 4 days and IVIG 100 mg/kg/day x 4 doses; followed by IVIG 2 gm/kg every 3 weeks x 4 doses and rituximab 3.75 mg/m^2 weekly x 2 doses[9] maintenance (both groups): calcineurin-inhibitor + mycophenolate mofetil + corticosteroids	Follow-up: 3 years Graft survival: group A = 50%, group B = 91% GFR: group A = 43 mL/min/1.7m^2, group B = 45 mL/min/1.7m^2
Brown et al.	18	PP x 8 sessions (1–3 days apart) maintenance: calcineurin-inhibitor + mycophenolate mofetil + corticosteroids	Follow-up: 5 years graft survival: 78% in responders mean creatinine: 1.5 mg/dL

American Journal of Transplantation 2011; 11: 896–906

Reducing DSA in Rejection Diminishes Allograft Loss

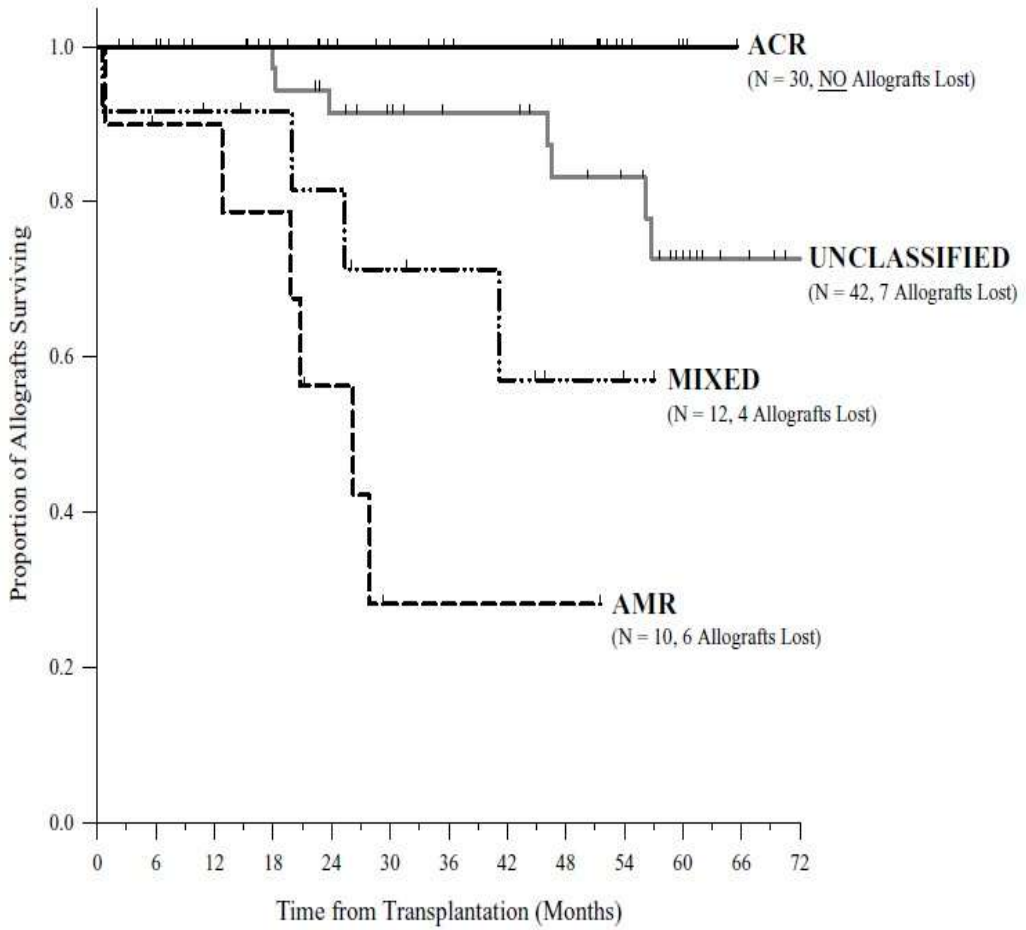


Figure 2: Death-censored allograft survival stratified by rejection type (ACR vs. mixed (p <0.001; log-rank), ACR vs. AMR (p < 0.001; log-rank) and unclassified vs. AMR (p < 0.001; logrank)).

American Journal of Transplantation 2009; 9: 1063–1071

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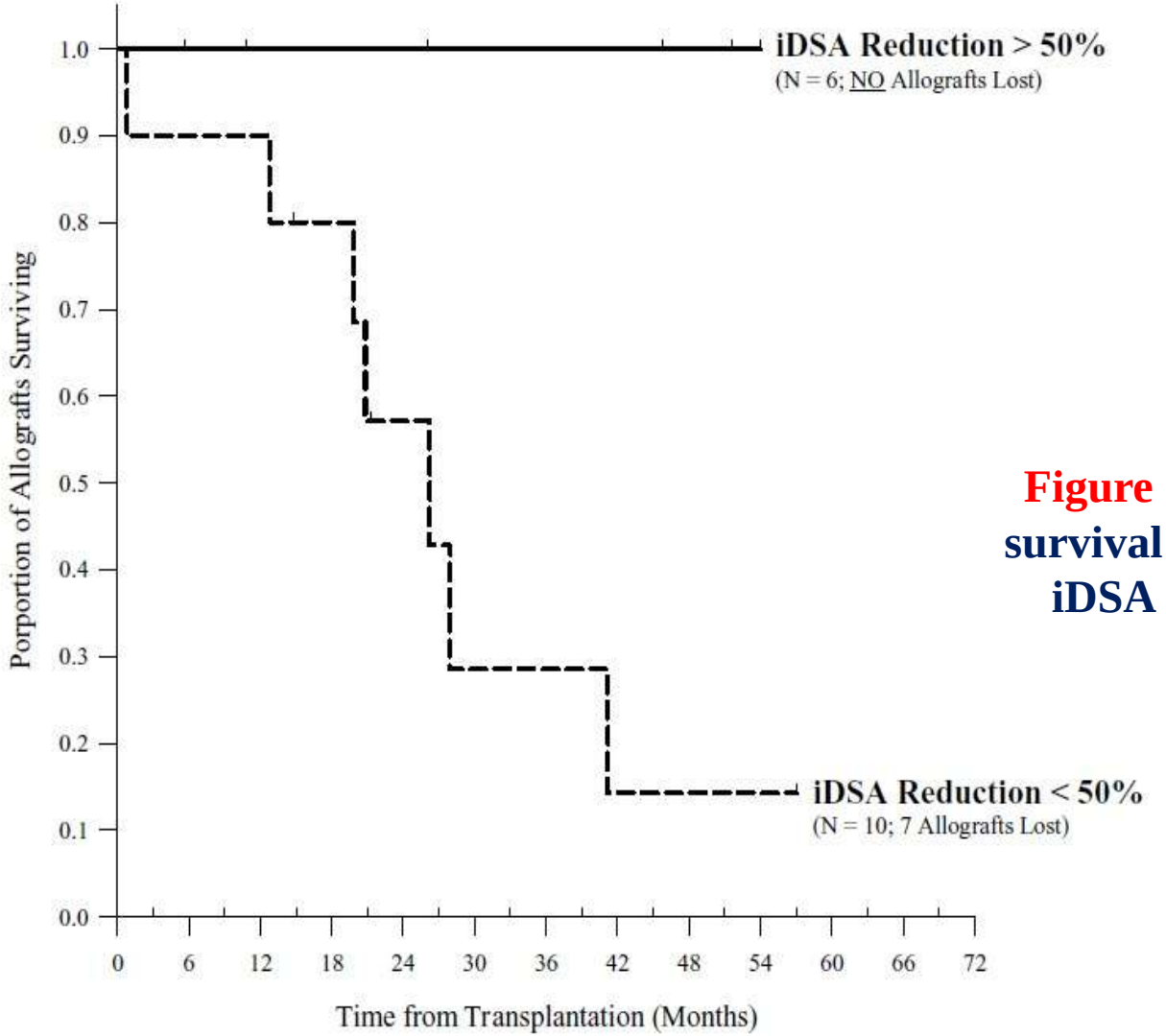


Figure 5: Death-censored allograft survival stratified by % reduction in iDSA at 14 days postbiopsy (p = 0.021; log-rank).

Everly et al. American Journal of Transplantation 2009; 9: 1063–1071

Table 2: Logistic regression analysis of risk factors for renal allograft loss

Variable	χ^2	p-Value	Odds ratio	95% CI
Recipient gender (male)	0.02	0.887	0.900	0.211–3.827
Recipient age at transplant	0.98	0.335	0.969	0.911–1.032
Recipient African American	5.70	0.030	6.533	1.203–35.478
Donor age	0	1.000	1.000	0.942–1.061
Donor African American	0.39	0.526	1.680	0.338–8.347
Deceased donor kidney transplant	1.02	0.314	2.109	0.493–9.019
AB mismatch	0.83	0.367	1.479	0.632–3.461
DR mismatch	1.31	0.266	0.443	0.106–1.859
CDC PRA current >20% and/or peak >50%	2.49	0.102	5.857	0.705–48.676
Repeat transplant	3.97	0.041	6.667	1.084–40.975
Delayed graft function	2.49	0.102	5.860	0.705–48.676
Banff grade ACR ≥IIA	0.24	0.631	0.659	0.120–3.612
DSA	6.09	0.017	6.600	1.392–31.283
C4d	5.67	0.016	7.800	1.468–41.418
Rejection reversal	0.36	0.536	0.567	0.094–3.408
Repeat rejection	1.04	0.285	2.785	0.425–18.226

Multivariate stepwise logistic regression

Variable	Coefficient (β)	Standard error	χ^2	p-Value	Odds ratio	95% CI
Intercept	−2.397	0.603	–	–	–	–
DSA	1.887	0.794	6.09	0.017	6.6	1.392–31.283

C4d = diffuse C4d by immunohistochemistry; CDC = complement-dependent cytotoxicity; PRA = panel reactive antibody. Delayed graft function is defined as the need for at least one session of dialysis during the first 7 days after transplantation.
$\text{Log}*(p/1-p) = -2.397 + 1.887 * \text{DSA}$.

Everly et al. American Journal of Transplantation 2009; 9: 1063–1071

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Table 1. Points Acquired for Various Parameters.	
Parameter	Points allocated
1. PRA > 50% with DSA	1
2. FCXM >100 MCS	1
3. FCXM > 200 MCS	2
4. DGF in first week	1
5. sCr rise > 50%	1
Following implementation of posttransplant monitoring, a preemptive treatment formula was developed that awarded points for PRA/DSA at time of transplant, preoperative FCXM levels and DGF and rapid rise in sCr occurring within the first week of transplant. Patients with ≥3 points received IVIG and plasmapheresis three times weekly for 2 weeks. Patients with 1-2 points were retested at a later date and rescored.	

IVIg + plasmapheresis 3 times per week For 2 weeks

Kimball et al. Clinical Transplants 2011

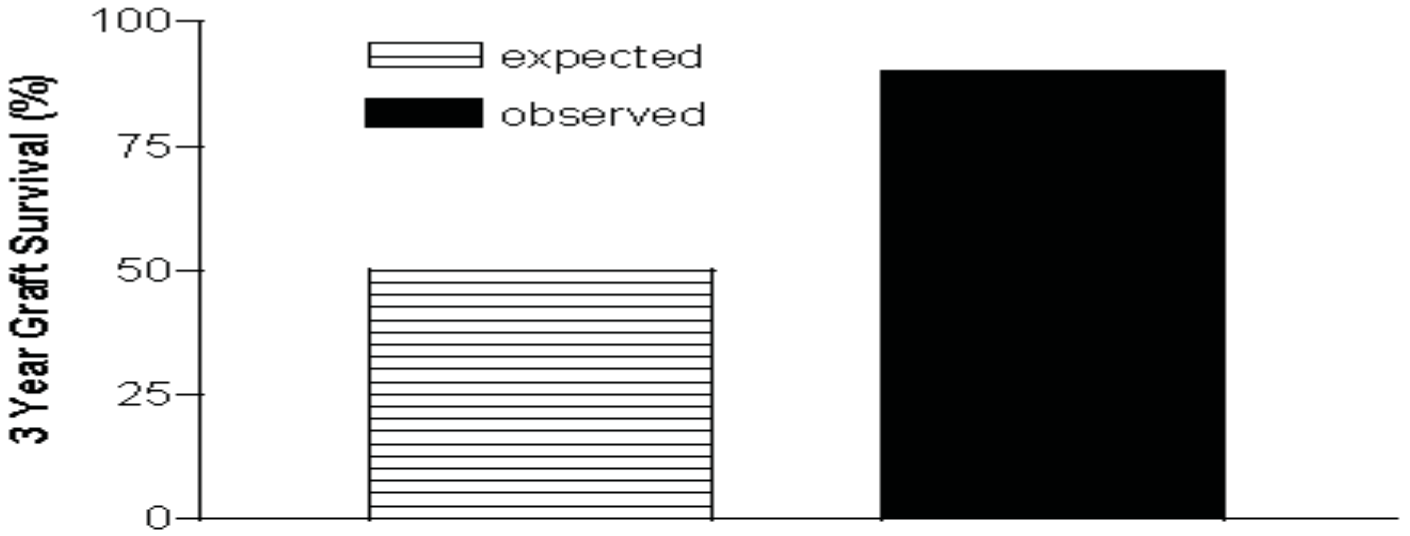
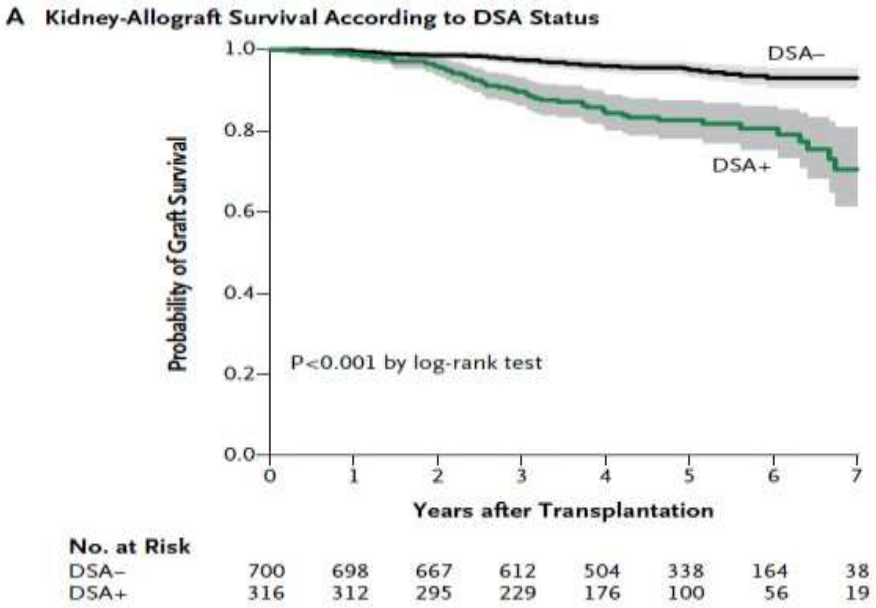
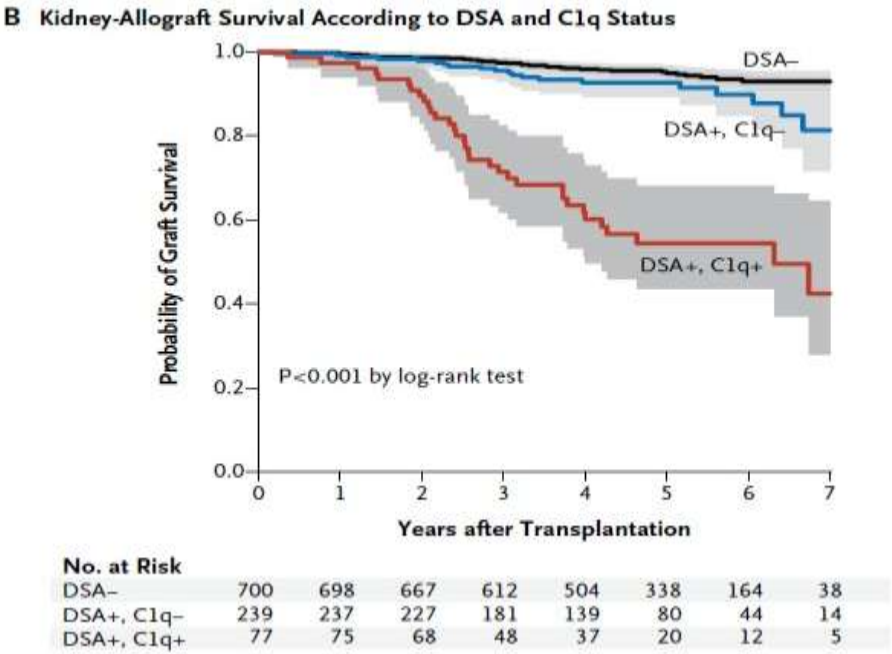


Figure 4. A comparison of 3-year graft survival among Group II patients before (expected) and after (observed) preemptive therapy was started. Forty patients with preoperatively positive FCXM and DSA were preemptively treated with IVIG and plasmapheresis if they received ≥ 3 points in an algorithm that gives points for specific parameters. Graft survival was significantly greater ($p<0.05$) among patients receiving preemptive treatment compared to historic outcomes among non-treated patients.

Kimball et al. Clinical Transplants 2011

Possible DSA Monitoring Time Points
Pre-Transplant, Day of Transplant
1 week
2 weeks
4 weeks
8 weeks





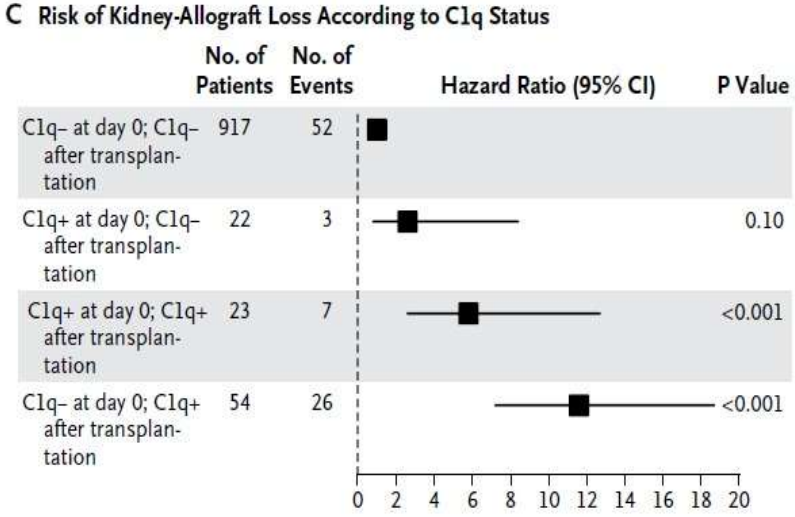


Figure 2. Kaplan–Meier Curves for Kidney-Graft Survival, According to Donor-Specific Anti-HLA Antibody Status after Transplantation.

Panel A shows the classic approach to determining the probability of graft survival, which is based on the presence or absence of donor-specific anti-HLA antibodies. Panel B shows our approach, which is based on the presence or absence of donor-specific anti-HLA antibodies and their C1q-binding capacity. Panel C shows the risk of graft loss according to C1q status at day 0 and after transplantation.

N Engl J Med. 2013 September 26, 369;13

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Phenotypic Features of Kidney-Allograft Injury, According to Anti-HLA Antibody Status after Transplantation

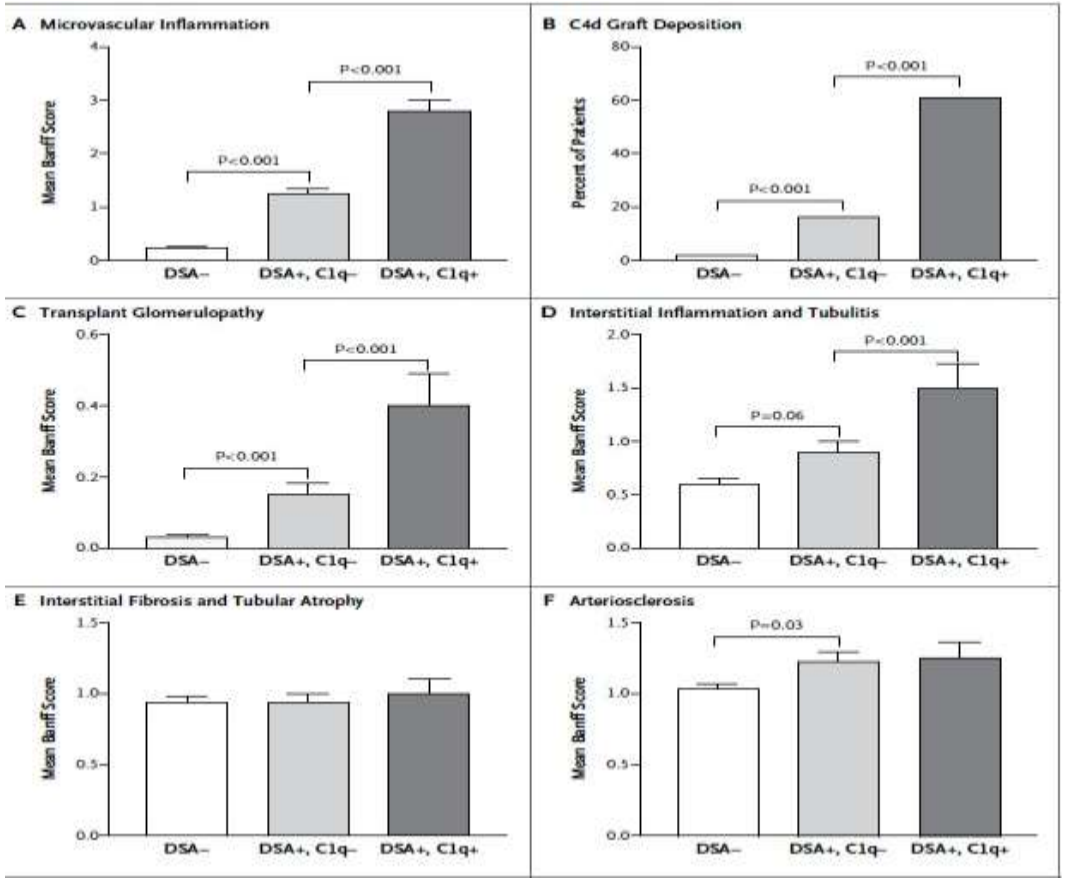


Figure1. Data are based on 1016 kidney-allograft biopsies performed in the first year after transplantation (845 at 1 year after transplantation and 171 during acute rejection in the first year). The score for microvascular inflammation (Panel A) is the sum of the Banff scores for glomerulitis and peritubular capillaritis. Each of these scores ranges from 0 to 3, with higher scores indicating more severe abnormality. The T bars indicate standard errors. DSA denotes donor-specific anti-HLA antibody

N Engl J Med. 2013 September 26, 369;13

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Monitoring DSA during the 1st year

Consensus Guidelines on the Testing and Clinical Management Issues Associated With HLA and Non-HLA Antibodies in Transplantation

Very high-risk (desensitized)	High-risk patients (DSA+, XM-)	Intermediate risk (only historic DSA)	Low risk (unsensitized, 1st Tx)
DSA & Bx in 1st 3 months	DSA & protocol Bx in 1st 3 months	Monitor DSA in 1st month	Monitor for DSA: - 3-12 months after Tx - If minimization - Non compliance - Dysfuntcion
Treatment if -Clinical or subclinical AR - DSA increase	Treatment if -Clinical or subclinical AR - DSA increase	If DSA+, Bx and: -Treat if AR - Monitor in 1 year if Bx-, but high IS	If DSA+, Bx and: -Treat if AR - Monitor in 1 year if Bx-, but high IS
	Persistent DSA and normal Bx: high IS		
	DSA and Bx- AS LOW RISK	DSA- AS LOW RISK	DSA- No monitor 1 year

Transplantation. Volume 95, Nuvember 1, January 15, 2013

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Monitoring DSA after the 1st year

Consensus Guidelines on the Testing and Clinical Management Issues Associated With HLA and Non-HLA Antibodies in Transplantation

>12 months postransplantation:

-Store 1 serum per year

-Test for DSA if:

-Relevant changes in IS
-Non compliance
-Dysfuntcion
-Transfer to other center

Risk factors for postransplant HLA antibodies

Crespo et al. 38 DSA in KT vs. 55 HLA no DSA vs 300 no antibodies.

DSA vs HLA no DSA

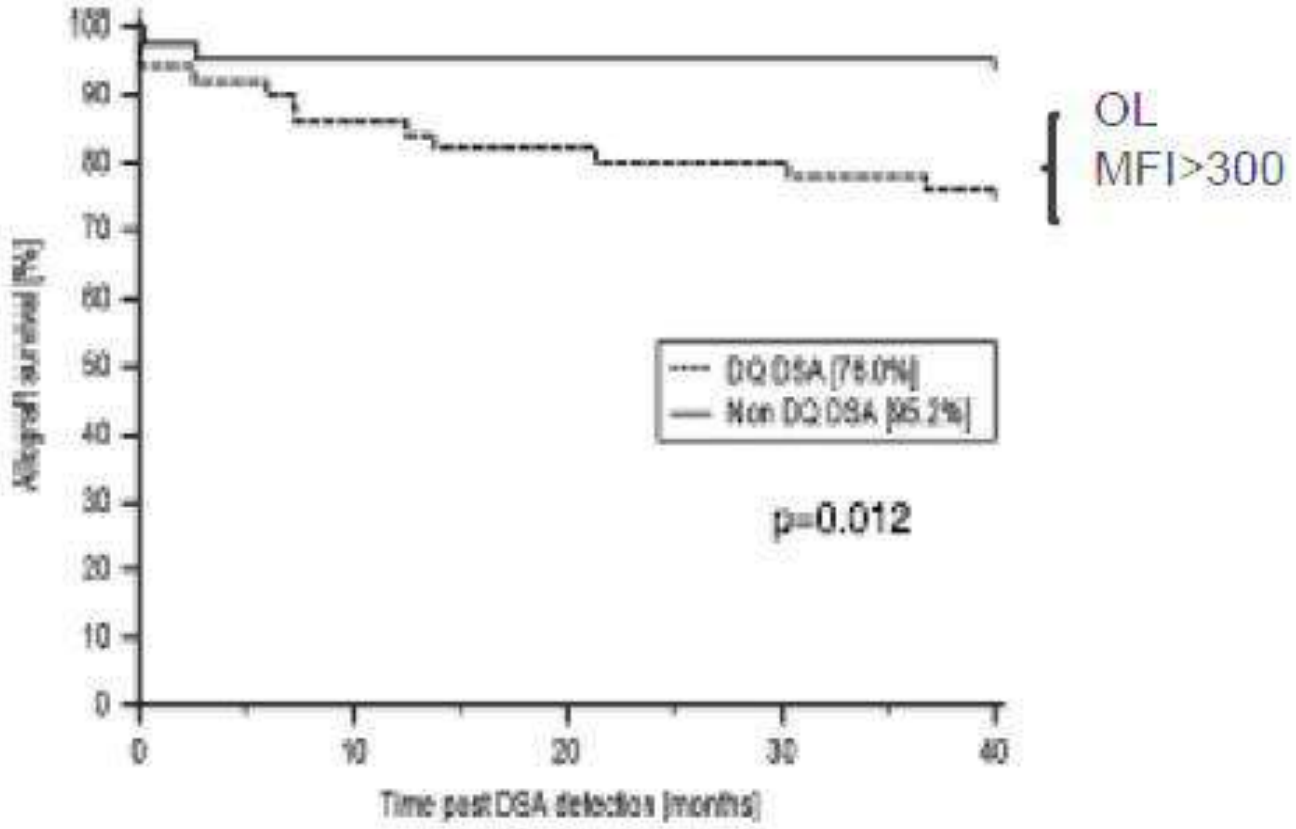
	OR	CI 95%		p
HLA DR mismatches	5.970	2.147	16.605	0.001
%$CD3^{-}CD56^{+}$ $NKG2A^{+}$ cells	1.041	1.007	1.076	0.018
Recipient female gender	3.690	1.192	11.363	0.024
Acute rejection	16.513	1.429	190.833	0.025
Pretransplant PRA CDC (%)	1.063	0.992	1.138	0.085
Recipient age at transplant	0.985	0.949	1.022	0.428
Retransplantation	0.927	0.285	3.018	0.900

Discrimination power, AUC (95%IC) 0.729 (0.641–0.817).

Crespo/ Pascual et al. American Journal of Transplantation 2015

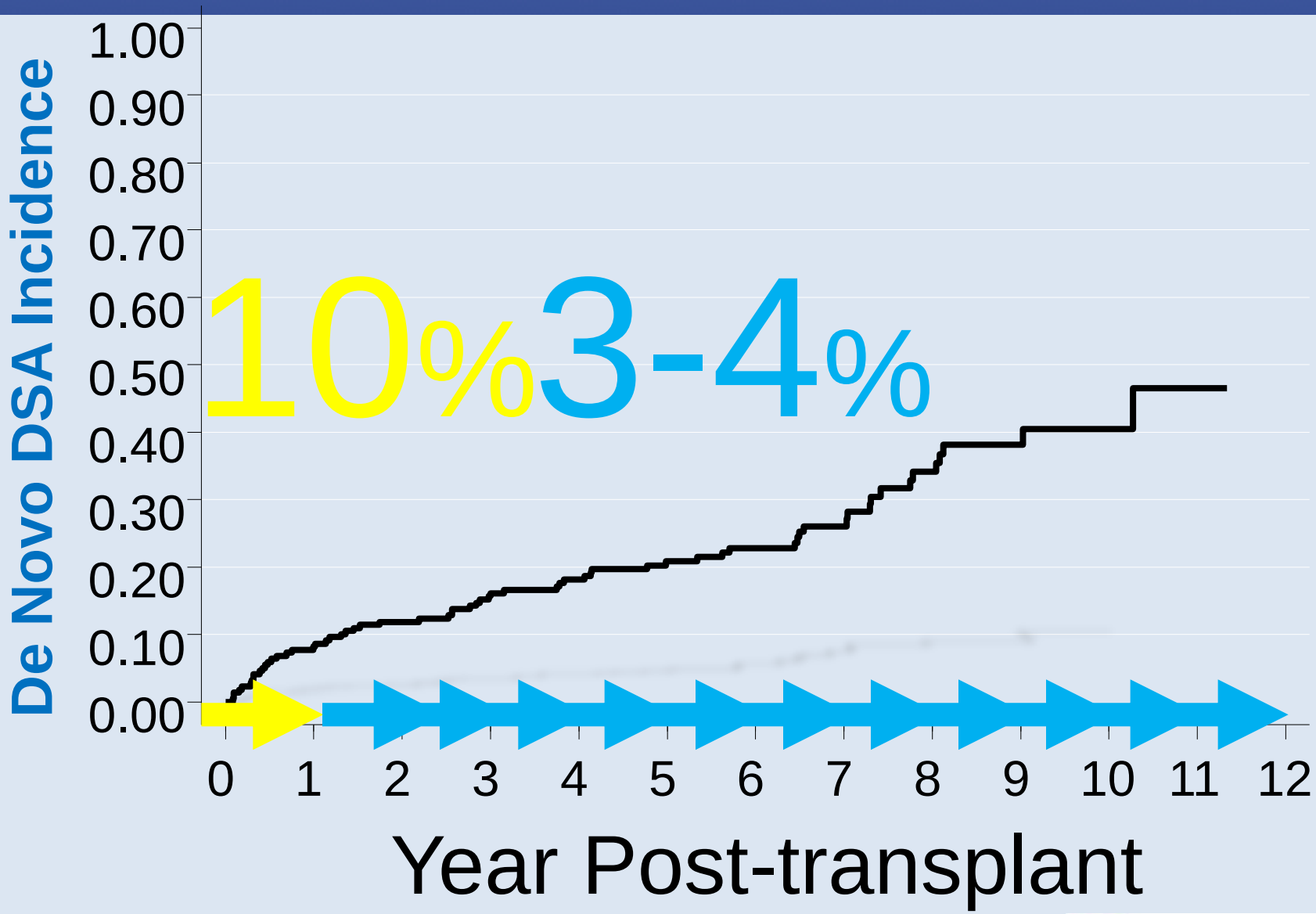
Postransplant anti-dq DSA and graft survival

Willicombe et al. 50 DSA DQ vs 42 DSA no DQ



Transplantation. Volume 94, Nuvember 2, July 27, 2013

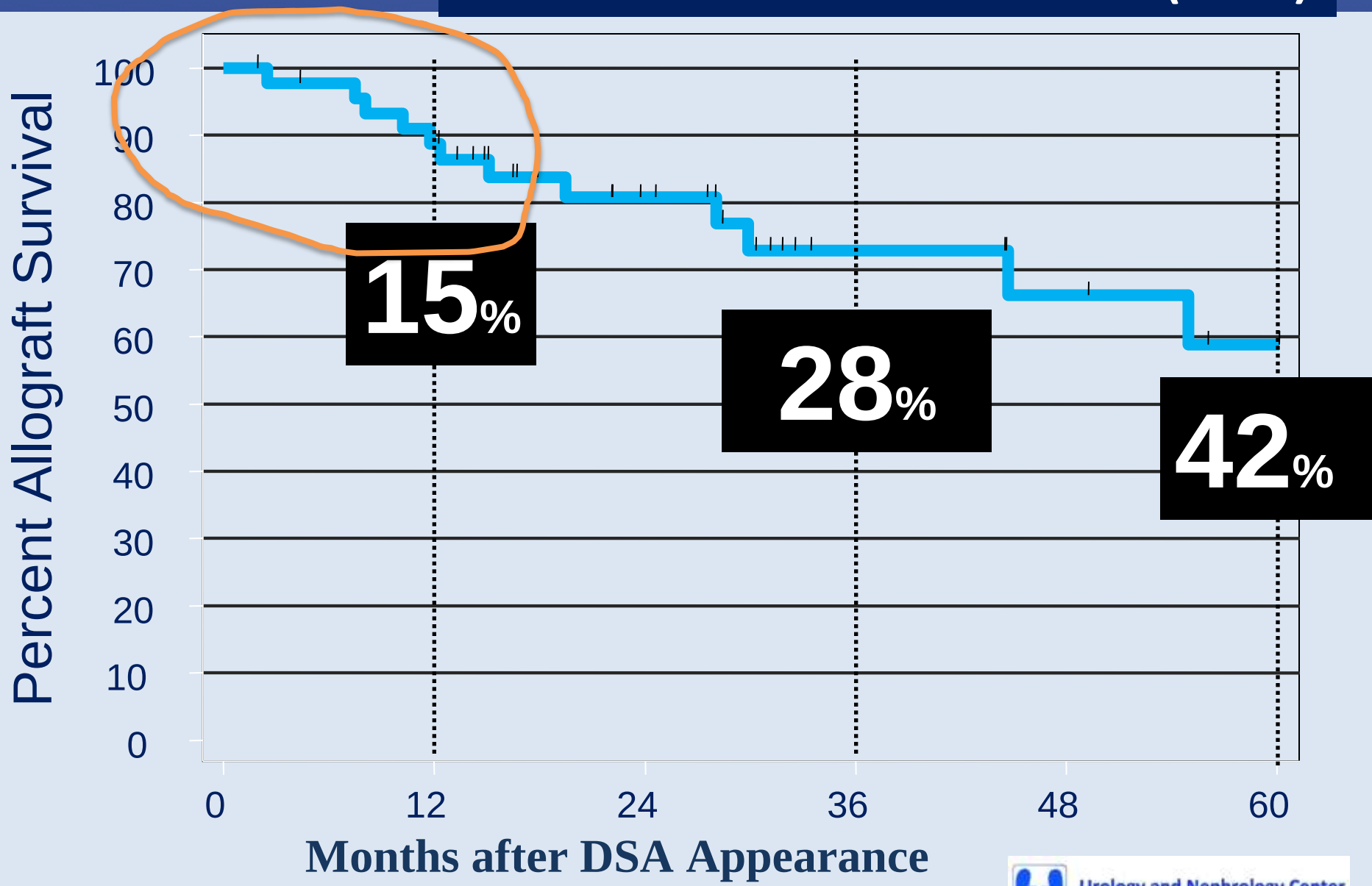
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Everly et al. Am J Transplant 2012.

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De novo anti-HLA DSA Positive (n=62)



Everly et al. Am J Transplant 2012.

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Monitoring Time Points	
Pre-Transplant	
~~1 week~~	
~~2 weeks~~	With for cause biopsy
~~4 weeks~~	
~~8 weeks~~	
6 months	
12 months	
Biannually after 1 year	

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Review

The Influence of Immunosuppressive Agents on the Risk of De Novo Donor-Specific HLA Antibody Production in Solid Organ Transplant Recipients

Jacqueline G. O'Leary, MD, MPH,[1] Millie Samaniego, MD,[2] Marta Crespo Barrio, MD,[3] Luciano Potena, MD, PhD,[4] Adriana Zeevi, PhD,[5] Arjang Djamali, MD,[6] and Emanuele Cozzi, MD, PhD[7]

(Transplantation 2016;100: 39–53)

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Table 1. Induction therapy:
A comparison of the risk of dnDSA

Reference	Study design	N	Follow-Up	Induction regimen	Maintenance regimen	dnDSA	*P*
rATG							
Brokhof et al, 2014[101]	Prospective Single center	114	3 y	rATG or BAS	TAC MMF Steroids	HR = 0.16* rATG vs BAS	0.003*
Huang et al, 2012[55]	Retrospective Cohort study Single center	145	1 y	rATG or BAS or None	CsA or TAC MPA Steroids	7.4% rATG 40% BAS 7.1% none	0.30
Alemtuzumab							
Todeschini et al, 2013[102]	Retrospective Matched control study Single center	48	2 y	ALEM or rATG/BAS	CsA or SIR MMF Steroids to day 7	57% ALEM 12.5% rATG/BAS	0.01
Rituximab							
Tydén et al, 2012[103]	Prospective Double-blind Multicenter	71	3 y	RITUX or Placebo	TAC MMF Steroids	3% RITUX 16% placebo	0.10
Bortezomib							
Ejaz et al, 2013[104]	Prospective Randomized 2 centers	40	1 y	rATG or rATG/RITUX or rATG/BORT or rATG/RIT/BORT	TAC MMF Steroids	30% rATG 30% rATG/RITUX 10% rATG/BORT 30% rATG/RITUX/ BORT	n/a

Asterisk indicates analyses were performed with multivariate analysis.
ALEM, alemtuzumab; BAS, basiliximab; BORT; bortezomib; n/a, not available; rATG, rabbit antithymocyte globulin; RITUX, rituximab; SIR, sirolimus; TAC, tacrolimus.

(Transplantation 2016;100: 39–53)

Table 2. Calcineurin inhibitor maintenance therapy: A comparison of the risk of dnDSA

Reference	Study design	Organ type	N	Follow-up	maintenance regimen	Univariate or multivariate* analysis		Use of Therapy according to presence/absence of dnDSA
						dnDSA	*P*	
Del Bello et al, 2014[47]	Retrospective Cohort study Single center	Liver	232	Median 36.5 mo	Various	n/a	n/a	CNI therapy was used[a] in 81% and 80% of patients with or without dnDSA, respectively
Kaneku et al, 2013[6]	Retrospective Cohort study Single center	Liver	749	1 y	CsA or TAC ± MMF/AZA ± Sirolimus ± Steroids	OR = 2.5 CsA vs TAC*	0.004*	
Cooper et al, 2011[42]	Retrospective Cohort study Single center	Kidney or kidney-pancreas	244	2 y	Various	n/a	n/a	TAC was used[a] in 77% of patients with dnDSA and 90% of patients with no dnDSA ($P = 0.009$)
Huang et al, 2012[55]	Retrospective Cohort study Single center	Kidney	145	1 y	CsA or TAC MPA Steroids	22% CsA 4% TAC	0.02	
Lachmann et al, 2006[130]	Prospective Cohort study Single center	Kidney	1043	>6 mo to 4 y	CsA or TAC ± MMF/AZA ± Steroids	35% CsA[b] 20% TAC[b]	0.05	
Hourmant et al, 2005[4]	Prospective Cohort study Single center	Kidney	1229	5 y	Various	—	—	CNI was used[a] in 93% and 95% of patients with or without dnDSA, respectively (n.s.)

Asterisk indicates analyses were performed with multivariate analysis.
[a]At the time the antibody results were obtained.
[b]Included both preformed and de novo DSA.
AZA, azathioprine; n.s., nonsignificant.

(Transplantation 2016;100: 39–53)

Table 3. Antimetabolite or purine synthesis inhibitor maintenance therapy: A comparison of the risk of dnDSA

Reference	Study design	Organ type	N	Follow-up	Maintenance regimen	Univariate or multivariate* analysis		Use of therapy according to presence/absence of dnDSA
						dnDSA	P	
Del Bello et al, 2014[47]	Retrospective Cohort study Single center	Liver	232	Median 36.5 mo	Various	n/a	n/a	MMF was used[a] in 90% of patients with dnDSA vs 71% of patients with no dnDSA ($P = 0.04$)
Kaneku et al, 2013[6]	Retrospective Cohort study Single center	Liver	749	1 y	MMF or none/AZA[b] ± CsA/TAC ± Sirolimus ± Steroids	OR = 1.00 MMF vs no MMF*	0.99	
Lachmann et al, 2006[130]	Prospective Cohort study Single center	Kidney	1043	>6 mo to 4 y	MMF or AZA or no antimetabolite CsA or TAC ± Steroids	39% AZA[c] 26% MMF[c] 19% none[c]	0.37	
Piazza et al, 2006[136]	Retrospective Cohort study Single center	Kidney	449	Not stated	MMF or AZA CsA Steroids	8% MMF 23% AZA	<0.0001	
Hourmant et al, 2005[4]	Prospective Cohort study Single center	Kidney	1229	5 y	Various	n/a	n/a	MMF was used[a] in 43%, 31% and 53% of patients with no antibodies, dnDSA or non-DSA ($P < 0.02$) AZA was used[a] in 31%, 43% and 23% of patients with no antibodies, dnDSA or non-DSA ($P < 0.02$)

Asterisk indicates analyses were performed with multivariate analysis.
[a]At the time the antibody results were obtained.
[b]363 patients received no induction, 9 patients received AZA.
[c]Included both preformed and de novo DSA.

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Table 4. mTOR inhibitor maintenance therapy: A comparison of the risk of dnDSA

Reference	Study design	Organ type	N	Time from transplant to Switch	Follow-up	Maintenance regimen	Univariate or multivariate* analysis: dnDSA	P
Kaneku et al, 2013[6]	Retrospective Cohort study Single center	Liver	749	—	1 y	SRL (n = 119) or no SRL (n = 630) at 1 y ± MMF/AZA ± CsA/TAC ± Steroids	OR = 0.66* SRL vs no SRL	0.36*
Early switch (<1 y) to mTOR inhibitor								
Liefeldt et al, 2012[54]	Prospective Randomized[a] Single center	Kidney	127	3-4 mo	Median, 3.5 y	CsA or CsA converted to EVR (months 3–4.5) MPA Steroids	HR = 2.67* EVR vs CsA	0.04*
Late switch (>1 y) to mTOR inhibitor								
Croze et al, 2014[18]	Retrospective Cohort study Single center	Kidney	270	Mean, 1.3 y	Mean, 3.8 y	TAC[b] switched to SRL[c] ± Steroids to month 3	HR = 2.4* SRL vs TAC	0.04*
Ruiz San Millán et al, 2014[137]	Retrospective Cohort study Single center	Kidney	35	Median, 69 mo (range, 3-375)	2 y	CNI switched to mTOR inhibitor ± MMF/AZA ± Steroids	8.6% mTOR inhibitor 0% controls (n = 10)	n.s.
Perbos et al, 2014[138]	Retrospective Case control study Single center	Various	131	Mean, 73 mo	1 y	CNI switched to EVR ± CNI[d] or CNI ± MMF (controls)	6.7% EVR 11.1% controls	0.39

Asterisk indicates analyses were performed with multivariate analysis.
[a] Post hoc analysis of patients randomized at a single center within a multicenter study.
[b] 17 patients received CsA instead of TAC.
[c] 9 patients received EVR instead of SRL.
[d] 59% of EVR-treated patients also received low-dose CNI.
EVR, everolimus; mTORi, mammalian target of rapamycin inhibitor; SRL, sirolimus.

(Transplantation 2016;100: 39–53)



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Table 5. Steroid maintenance therapy: A comparison of the risk of dnDSA

Reference	Study design	Organ type	N	Follow-up	Maintenance regimen	Univariate or multivariate* analysis		Use of Therapy according to presence/absence of dnDSA
						dnDSA	*P*	
Del Bello et al, 2014[47]	Retrospective Cohort study Single center	Liver	232	Median 36.5 mo	Various	n/a	n/a	Steroids were used[a] in 19% of patients with dnDSA 19% and 44% of patients with no dnDSA (*P* = 0.02)
Kaneku et al, 2013[6]	Retrospective Cohort study Single center	Liver	749	1 y	Steroids or no steroids ± CsA/TAC ± MMF/AZA ± Sirolimus	OR = 0.67* steroids vs no steroids	0.23*	
Hoshino et al, 2012[141]	Prospective Cohort study Single center	Kidney	72	1 y	Clonal depletion Steroids ± CNI, MMF or sirolimus	ARR = 0.92 per 2.5 mg/day increase in steroid dose	0.03	
Delgado et al, 2009[65]	Prospective Double-blind Randomized Single center	Kidney	37	≤5 y	TAC MMF Steroid withdrawal at day 7 (n = 21) or standard steroids (n = 16)	0% steroid withdrawal 6.3% standard steroids	0.43	
Lachmann et al, 2006[130]	Prospective Cohort study Single center	Kidney	1043	>6 mo to 4 y	Steroids or no steroids CsA or TAC ± MMF/AZA	27% steroids[b] 33% no steroids[b]	0.44	
Hourmant et al, 2005[4]	Prospective Cohort study Single center	Kidney	1229	5 y	Various	n/a	n/a	Steroids were used[a] in 22%, 40% and 43% of patients with no antibodies, dnDSA or non-DSA (n.s.)

[a]At the time the antibody results were obtained.
[b]Included both preformed and de novo DSA.
ARR, adjusted risk ratio.

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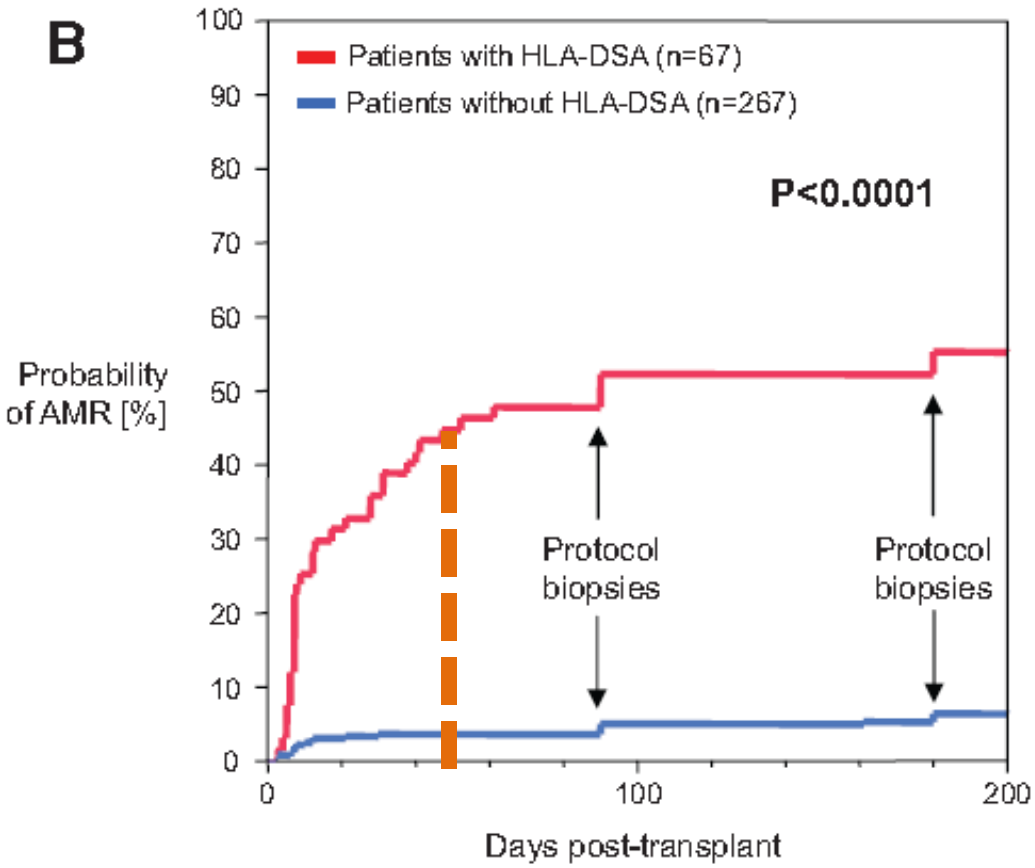
Overall, in at risk individuals we have to maintain adequate immunosuppression, establish appropriate DSA monitoring protocol and to enforce adherence

Conclusion

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Nearly All transplant patients can benefit for some degree of monitoring …

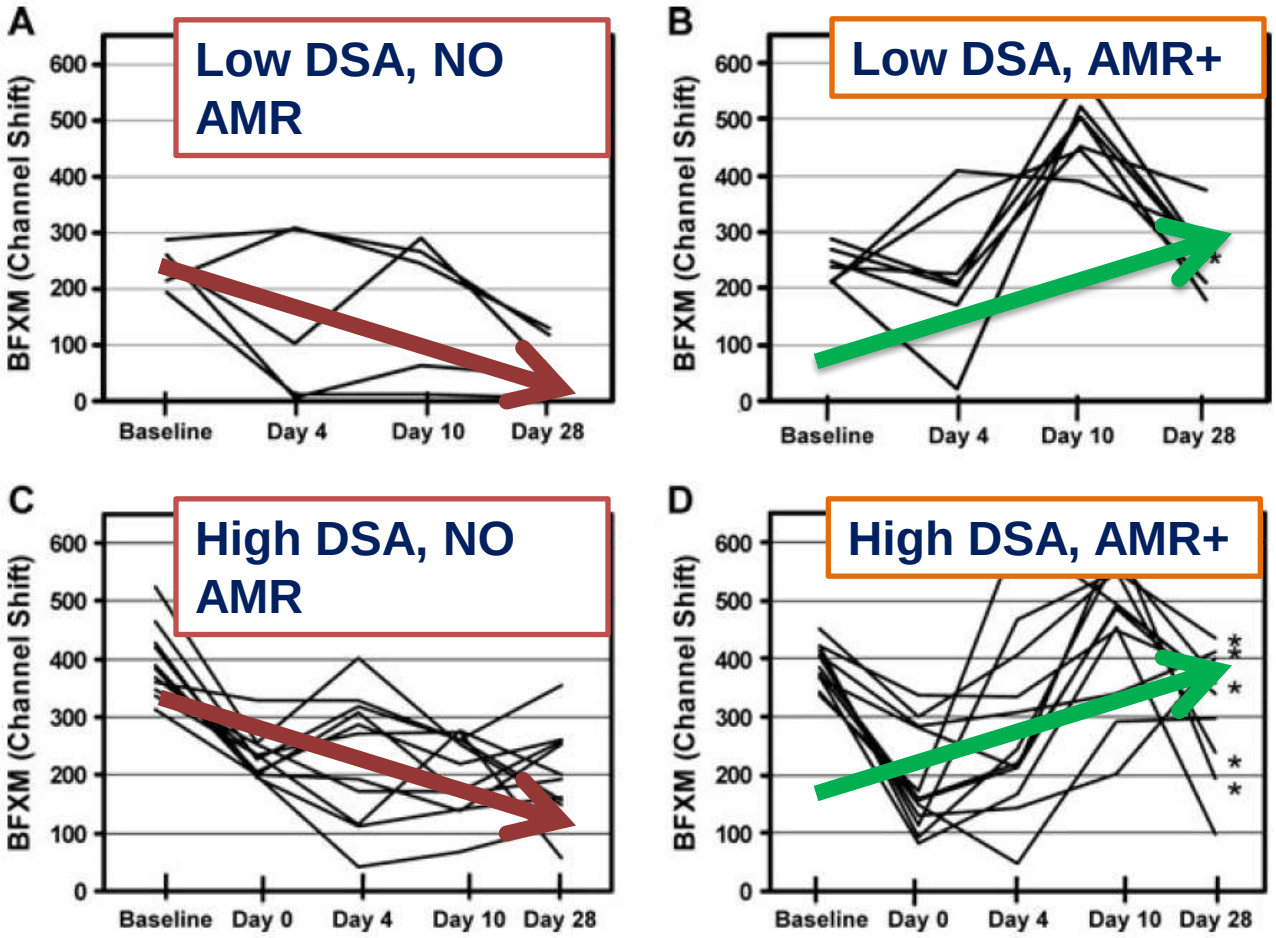
AMR Appears Early



Amico et al. Transplantation 2009;87:1681

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Monitoring Early Identifies Those AT RISK Of AMR



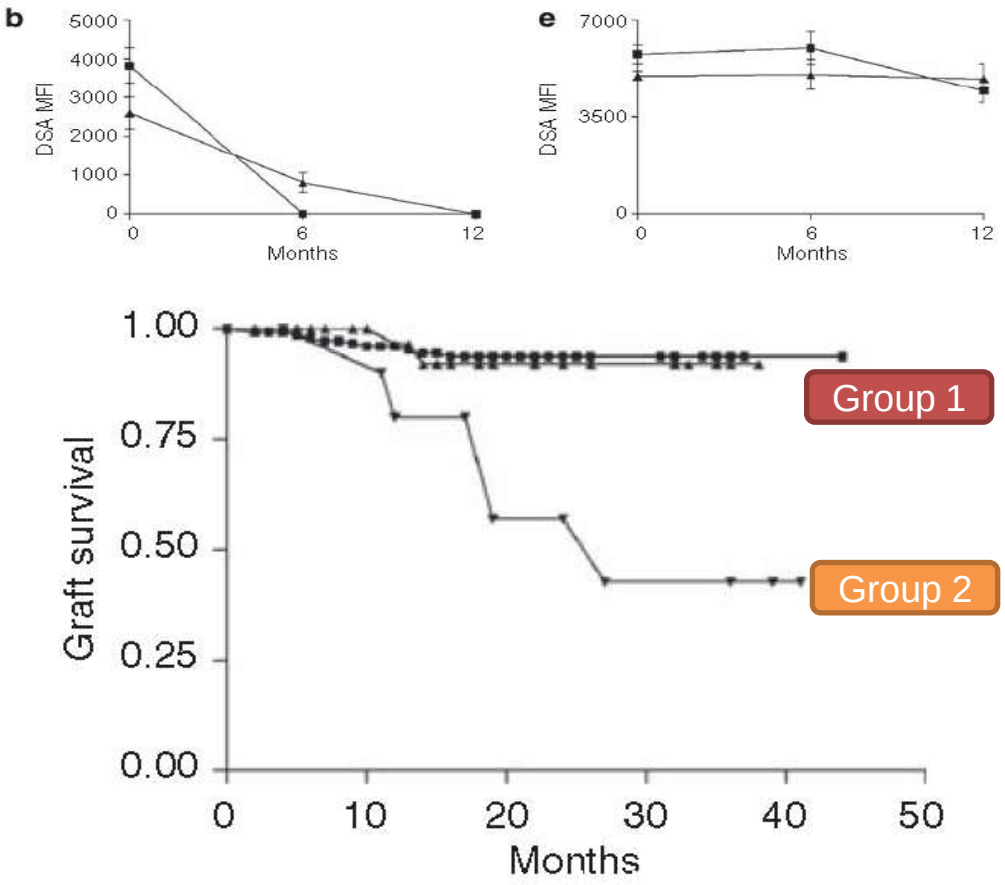
Burns et al. Am J Transplant 2008;8:2684

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Monitoring Late Identifies those AT RISK of Failure

Group 1

Group 2



Monitoring Time Points

Pre-Transplant
1 week
2 weeks
4 weeks
8 weeks
6 months
12 months
After 12 months, annually

With for cause biopsy

How to face the sensitized patient?

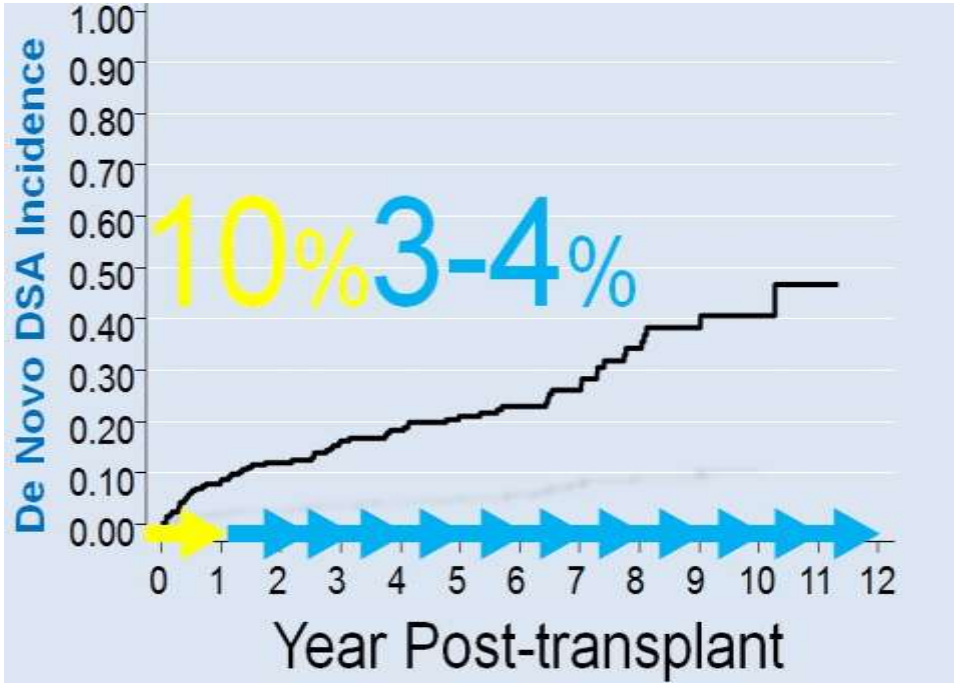
Combining all possibilities(related living donor, paired, desensitization, sharing programs).

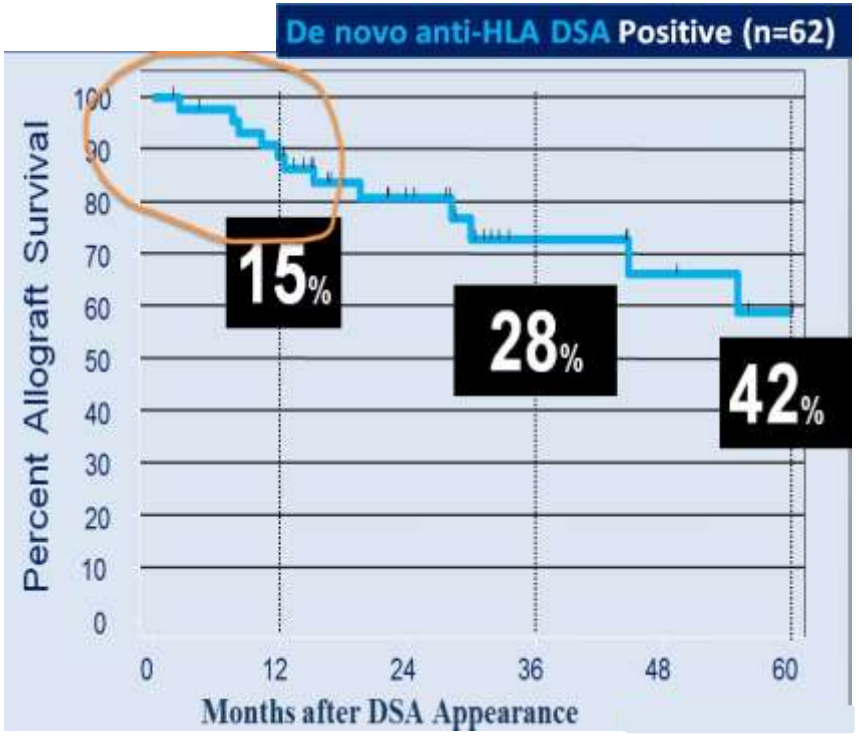
-Detailed evaluation of the HLA sensitization.

-The most efficacious DS strategy combines PE, low dose IvIG& rituximab.

-For patients on the waiting list, programs sharing donors help but “as much as 40% of hypersensitized patients will not find a compatible donor”.

De novo anti-HLA DSA appears at any time post transplant & Identifies those AT RISK of Failure



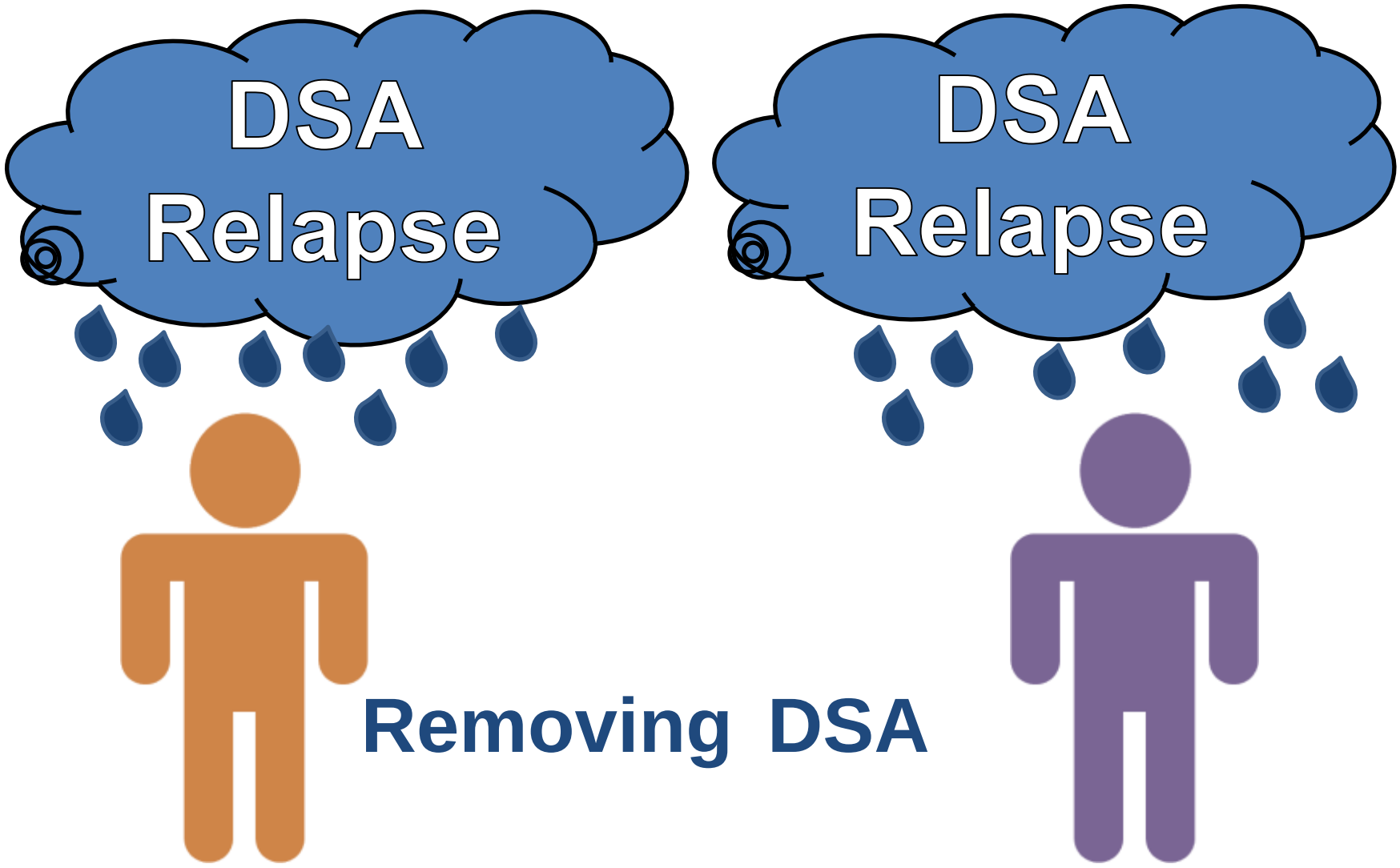


Everly et al. Presented at American Transplant Congress 2011, Philadelphia
Everly et al. Am J Transplant 2012

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Monitoring Time Points

Pre-Transplant	With for cause biopsy
6 months	
12 months	
Annually after 1 year	



Improves Outcomes

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Thank YOU